César Pérez-Cruzado

Doctoral Thesis

MODELS FOR ESTIMATING BIOMASS AND CARBON IN BIOMASS AND SOILS IN *Pinus radiata* (D. Don), *Eucalyptus globulus* (Labill) AND *Eucalyptus nitens* (Deane & Maiden) Maiden PLANTATIONS ESTABLISHED IN FORMER AGRICULTURAL LANDS IN NORTHWESTERN SPAIN

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TESIS DOCTORAL

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CÉSAR PÉREZ-CRUZADO

INGENIERO DE MONTES

Memoria para optar al grado de Doctor realizada bajo la dirección de los Doctores del Departamento de Producción Vegetal y Edafología y Química Agrícola de la Universidad de Santiago de Compostela:

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El Dr. D. Roque Rodríguez Soalleiro, Profesor Titular del Departamento de Producción Vegetal de la Universidad de Santiago de Compostela y Dr. D. Agustín Merino García, Profesor Titular del Departamento de Edafología y Química Agrícola de la Universidad de Santiago de Compostela, informan:

Que la memoria titulada "Models for estimating biomass and carbon in biomass and soils in *Pinus radiata* (D. Don), *Eucalyptus globulus* (Labill) and *Eucalyptus nitens* (Deane & Maiden) Maiden plantations established in former agricultural lands in northwestern Spain", que para obtener el grado de Doctor Ingeniero de Montes presenta D. César Pérez Cruzado, ha sido realizado bajo nuestra dirección. Considerando que el trabajo está finalizado, y es materia de tesis, autorizamos su presentación.

Y para que así conste a los efectos oportunos, firmamos la presente en Lugo a 22 de Junio de 2011.

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0. Index and abstract

0.1. Index

0.2. General abstract

Climate change is one of the most serious environmental problems nowadays. This has been brought about by the so-called greenhouse effect, caused by the huge release of greenhouse gases, particularly CO_2, from the burning of fossil fuels. Energy sectors contribute most to climate change, although land use and land use change also contribute greatly to the greenhouse effect. Despite the observed trend towards deforestation in tropical zones, land use changes in Europe and North America are tending towards an increase in forest cover. This was accelerated in Europe by the implementation of a policy encouraging afforestation of former agricultural land (EEC 2080/92), which led to afforestation of large areas in northern Spain between 1992 and 2006. This usually involved planting fast growing species (*Eucalyptus globulus* Labill., *Eucalyptus nitens* (Deane & Maiden) Maiden and *Pinus radiata* (D. Don)) on former pasture land.

International agreements on global C emissions allow the countries involved to compensate for the release of CO_2 by providing C sinks. However, there is a large degree of uncertainty associated with the methods used to estimate the amounts of carbon sequestered, mainly when evaluating some compartments of C sinks, even in steady state systems. The process is more complicated when the land use under study has not reached equilibrium, and additional studies are required to evaluate the effects on C stocks.

Although estimation of the carbon density in tree biomass is simple, it is much more difficult to estimate changes in carbon density in soils. Most carbon dynamics studies are based on modelling approaches, and studies based on empirical data are required for more consistent assessments, and to provide information for validating eco-physiological model predictions or for developing empirical models. However, direct measuring is time consuming and the results are highly variable and do not always enable significant conclusions to be reached. Changes in soil carbon are difficult to estimate because they occur relatively slowly, and some external variables may also affect the final estimation. Chronosequence sampling combined with pairwise comparison of plots may be a useful technique for translating spatial differences into temporal differences, while also correcting local tendencies in the measured plots, thus enabling estimation of the changes in the variable of interest at landscape level.

Carbon in trees is usually estimated from biomass equations, which are supposed to be more useful for biomass estimation than biomass expansion factors. However, estimation of the dry mass of the sample trees used in developing models may not be very accurate. Moreover, the increasing interest in harvesting crown fractions and in carrying out ecological and nutritional studies, leads to the need for more accurate models. The study of crown variables as explanatory variables in biomass equations may lead to the development of more accurate models than those based exclusively on stem variables.

Product yield is usually expressed in terms of harvested or produced wood volume, whereas bioenergy substitution and carbon stocks are expressed in terms of energy, or tonnes of oil

equivalent. This depends on the transformation procedure, which finally leads to estimations from stand volume or biomass by the application of bioenergy production factors. Relating each of these forms of energy or carbon directly by the use of specific models may avoid concatenating errors in the estimation, and at the same time enable comparison of several management alternatives or the use of particular species for energy purposes.

The first step in estimating changes in soil organic carbon (SOC) after land use change is to examine changes in total SOC. However, the stability of SOC compounds varies greatly, and traditional techniques for describing these processes are time consuming and expensive. The development of inexpensive and rapid alternative methods for assessing changes in soil stability is a key factor for large scale or high temporal resolution studies.

When evaluating the whole forest sector in terms of climate change mitigation, carbon accumulation in wood products and the associated dynamics must be considered, although it is still not clear how this pool will be accredited in international emissions agreements. The most commonly used techniques are those based on mechanistic models, e.g. the CO_2Fix model, which enables estimation of the mitigatory effect of several management alternatives by considering all compartments in which carbon can be stored. However, few studies have been carried out to validate the results obtained with this model in southern Europe.

The aim of this doctoral thesis is to discuss modelling and estimation of C accumulation in forest systems, particularly forest plantations. The study focused on the three levels at which carbon can be estimated: tree, stand and landscape level. The study involved evaluation of a specific dynamic process, i.e. the afforestation of former pasture land in northern Spain. The main research questions addressed were thus related to this issue, with the aim of improving the tools and models used to quantify carbon stocks and carbon changes resulting from land use change. The study was carried in the temperate forest of Atlantic southern Europe, one of the most productive timber production areas in Europe.

The experimental design and methods used to collect data are summarised in the following points, corresponding to different chapters of the thesis.

- ✓ A network of 120 paired plots (former pasture land-new plantations of different ages) was established to construct three well-replicated chronosequences of the most common tree species in Atlantic temperate areas. The paired plots represented the original land use (pasture) and forest plantations established on this land. In each of the chronosequences, carbon density was estimated in aboveground biomass, litter and mineral soil to a depth of 30 cm. Changes in carbon in mineral soil and litter were evaluated by non parametric analysis, whereas in living biomass, carbon was calculated by regression analysis. The effect of rotation age on C stock for the average trends in the measured plots was also assessed.
- ✓ Forty specimens of *E. nitens* were felled and completely fresh weighed. The tree biomass was divided into the following components: wood, bark, thick branches, thin branches, twigs, leaves and dead branches along the stem. Intensive sampling was carried out along

the stem to evaluate the effect of sampling intensity on dry mass estimation, by ratio type estimators. Two different methods were compared: complete fresh weight and dry mass estimation using disks sampled along the stem (*CW*), and complete stem cubication and partial fresh weight for dry mass and average basic density estimation (*PW*). Moreover, the usefulness of crown variables for describing biomass crown biomass fractions was assessed. The utility of the models developed for estimating changes in biomass fractions proportion in aboveground biomass was assessed for different dimensional classes.

- ✓ Measurements made in of 15 additional plots for *E. globulus* and 36 for *E. nitens* established on forest land were used to develop static growth models for these species. The threshold density limit derived from self-thinning and harvesting were considered for model development, and enabled evaluation of technical and silvicultural limitations for the single stem rotation. The models enabled evaluation of the effect of two different initial stocking on bioenergy production in several different ways, as well as carbon accumulation for the two eucalypt species studied.
- ✓ Several soil samples were collected in the *E. globulus* and *P. radiata* chronosequence plots, to represent the average trends for these species, and were analysed by calorimetry and thermal analysis, as novel techniques to elucidate how afforestation affects the nature of soil organic matter (SOM) and soil microbial metabolism. The results were compared with those obtained by solid state nuclear magnetic resonance, often used to study SOM. The application of both techniques enabled estimation of the nature of changes in SOC following land use change, and highlighted the usefulness of calorimetry and thermal analysis for distinguishing soil organic compounds.
- ✓ The CO_2Fix mechanistic model was parameterized with data obtained in the abovementioned plots, and with additional measurements regarding forest industry sector products fluxes and performance. Detailed measurements of all compartments estimated by the CO_2Fix model in the 120 paired plots (see above) enabled validation of the CO_2Fix model estimations for the conditions under study. Detailed wood industrial sector data were obtain for parameterizing the wood products module. This, together with previously developed yield models and hypotheses regarding the lifespan of product, was used to evaluate the effects of two different management alternatives for the species considered, in relation to climate change mitigation.

Regarding the changes in C after land use change from pasture to forest plantations (Chapter II), the mean rates of C sequestration (biomass and soil) estimated throughout the rotation ranged between 8.7 and 14.6 Mg C ha^{-1} yr^{-1} (*Eucalyptus nitens* > *Eucalyptus globulus* > *Pinus radiata*), and the contribution of the soil (litter plus mineral soil) ranged from 8 to 18% (*Eucalyptus nitens* > *Pinus radiata* > *Eucalyptus globulus*). The humid temperate climate and the sandy loam texture of the soils favoured large losses of SOC from the uppermost mineral soils during the 10 years following afforestation. The losses were derived from the large content of SOM in the pasture soils in the region. The higher losses of SOC from the pine soil (26% of initial SOC compared with loss

of 19.5% of initial the SOC from soil under eucalypts) were attributed to the lower transfer of organic C to the mineral soil, as a result of the lower litter decomposition rate and the lower belowground litter input from associated vegetation. The latter factor was attributed to differences in shading provided by the different species, which finally leads to different microclimatic conditions in the plantations. The climate also favoured the rapid development of tree biomass and subsequent C sequestration in biomass and soils.

In the study of the effects controlling the SOC accumulation, soil carbon losses from the upper mineral soil layers in the plots with the highest site indexes were lower and recovery of the initial contents took place faster than in the other plots. The initial carbon content during the previous land use was a significant factor determining the changes in SOC following land use change. The initial carbon losses and later recovery of initial carbon contents were highest in plots in which the initial soil carbon content was highest. This effect was significant for both species of *Eucalyptus*, but not for *P. radiata*, possibly because of the death of herbaceous vegetation in *Pinus* plantations in the first years after land use change.

The results of non-parametric analysis of average trends enabled evaluation of the effect of different rotation ages on C stock and accumulation rate for the three species studied and the compartments considered. The C sink capacity of forest plantations can be maximized by lengthening the rotation and adopting suitable management strategies for each species. This is especially important in plantations in which the high intensity of harvesting may prevent accumulation of SOC in the long term.

In Chapter III, the intensive sampling considered for the stem component enabled estimation of the effect of sampling intensity and initial sampling point in the stem on the accuracy and bias in estimation of stem wood dry mass by ratio type estimators and systematic sampling. For both methodologies considered (*CW* and *PW*), the residues were plotted against sampling intensity, and thresholds were established to keep the relative error below 10%. The increases in moisture content and basic density along the stem explained the serious risk of dry mass overestimation when systematic subsamples were considered. Of the methods considered here, the *CW* approach produced better results for the largest dimensional class than the *PW* method for a similar sampling intensity. This is important because the *PW* method is usually used for large trees in which complete weighing is time-consuming.

The results clearly show the trends in relative errors derived from measurement of the bottom disk or the bottom log, considered by default as the first section that should be measured. This resulted in overestimations in the *CW* method and underestimations in the *PW* method. If systematic sampling is used, it is advisable to establish the sampling intensity before randomizing the position along the stem of the first disk or log to be measured. In the case of the *PW* method, it is not recommended to take only one sample log per tree, and the sample should be split along stem in an attempt to represent the average basic density, which usually occurs at a relative height of 30-35% along the stem.

The biomass equations were fitted by seemingly unrelated regression, with corrections for heteroscedasticity carried out by weighted fitting. Diameter at breast height was the best explanatory variable, and the inclusion of height did not improve the accuracy of estimation, except for the wood component. The inclusion of crown variables improved the predictive ability for crown fractions, increasing the accuracy of estimating thick branches (by 10.8%), twigs (by 19.1%) and leaves (by 17.3%). The biomass of each fraction decreased in the following order: wood > bark > thick branches > dead branches along the stem > leaves > thin branches > twigs. The changes in these percentages with diameter classes and the predictive ability of the fitted equations were also studied.

Development of stand level models for estimating biomass yield, total energy and carbon sequestration in *Eucalyptus globulus* and *Eucalyptus nitens* plantations is described in Chapter IV for first rotation stands established at the usual range of initial forest densities in south-western Europe. The timber volume, total aboveground biomass, logging residue biomass, crown biomass, carbon in aboveground biomass and soil organic layer, energy in aboveground biomass, energy in logging residue biomass and usable cellulose yield were represented in the form of isolines (taking mortality into account) and plotted against dominant height.

These variables were calculated and compared with previously published data on two silvicultural options for short rotation forestry, one destined for bioenergy production and the other consisting of the standard silviculture regime applied to both species in southern Europe, considering the average site index for each. Yield levels were higher in *Eucalyptus nitens* than in *Eucalyptus globulus* for all variables, because of faster diameter increment at similar densities. The total yield in terms of biomass was 13.9-14.6 Mg ha^{-1} yr^{-1} for *Eucalyptus globulus* and 20.4-21.5 Mg ha^{-1} yr^{-1} for *Eucalyptus nitens*. The energy in aboveground biomass ranged between 233-245 GJ ha^{-1} yr^{-1} for *Eucalyptus globulus* and 345-364 GJ ha^{-1} yr^{-1} for *Eucalyptus nitens*; the carbon accumulation rate in aboveground biomass and soil organic layer was 6.9-7.2 Mg ha^{-1} yr^{-1} for *Eucalyptus globulus* and 12.7-13.5 Mg ha^{-1} yr^{-1} for *Eucalyptus nitens*, and usable cellulose was 5.7-5.9 Mg ha^{-1} yr^{-1} for *Eucalyptus globulus* and 9.0-10.1 Mg ha^{-1} yr^{-1} for *Eucalyptus nitens*.

Simulated rotations were longer than the average lengths considered for short rotation woody crops. This has some positive effects, such as higher wood:bark ratios and larger average tree sizes. It was found that 50% increments in the initial density result in only marginal increments in biomass and usable cellulose yields. The density management diagrams constructed can be used as a powerful tool for management of eucalypt plantations with a multipurpose objective (pulp or solid timber production, fossil fuel substitution or carbon sequestration).

The application of calorimetry and thermal analysis as novel techniques to elucidate how afforestation affects the nature of SOM and soil microbial metabolism is described in Chapter V. The techniques were applied to study the SOM dynamics in two stands afforested with *Pinus radiata* and *Eucalyptus globulus*, established on pastures in a humid temperate region. Results of thermal analysis and calorimetry were compared with those obtained by solid state nuclear magnetic resonance, which is often used to examine changes in chemical SOC stability.

The application of differential scanning calorimetry and solid state nuclear magnetic resonance revealed that the SOM comprised carbohydrates, carbonyl/carboxyl groups, aliphatic components and aromatic carbon in the first years of the rotation. All these fractions became degraded after afforestation. The degradation was monitored by calorespirometry, which provided the calorespirometric ratio of the soil basal metabolism, together with the active biomass and the metabolic quotient. These indexes proved to be sensitive parameters that provided information about changes in the pattern of microbial metabolism in response to changes in the nature and redox state of the carbon substrates, demonstrating degradation of the aromatic and aliphatic organic matter fraction. The techniques were able to distinguish differences in SOM dynamics in the two types of stands, attributable to the different development of understory vegetation and litter composition.

A comprehensive study was carried out considering the carbon sink effect in biomass, soil and wood products, the substitutive effect of bioenergy, and particular conditions of the forest industry in southern Europe, as described in Chapter VI. The CO_2Fix model was parameterized by using the yield models, leaf and branch turnover ratios derived from the plots established and region-specific parameters for products and biomass. Validation was carried out for the biomass and the soil module with plot-specific climate data obtained from a network of 120 plots. Results showed strong bias in SOC estimation, which was attributed to overestimation of the decomposition rates in soil compartments. Slight bias in the carbon biomass estimation was also observed when yield models specific for forest land were used to simulate afforestation on former pasture land.

As regards the sensitivity, the Yasso model was found to be strongly robust to leaf, root and branch turnover. The woodchip production alternative yielded higher carbon stock in biomass and products, as well as in bioenergy substitution effect, than the sawn-wood production alternative. Nevertheless, the sawn-wood alternative was the most effective as regards the carbon stock in the soil. Site index had an important effect for all species, alternatives and compartments, and the mitigating effects on climate change increased with site index. Harvesting clearcutting and thinning slash for bioenergy use led to a slight decrease in the soil carbon equilibrium, but significantly increased the mitigating effect through bionergy use.

0.3. Resumen

El cambio climático es hoy en día uno de los problemas ambientales más preocupantes a nivel mundial. Es causado por el denominado efecto invernadero, debido en último término a la liberación de cantidades ingentes de gases de efecto invernadero, particularmente CO_2. El sector energético es el que actualmente más contribuye al efecto invernadero, aunque el cambio de uso del suelo debido a la deforestación también tiene una elevada importancia. A pesar de las tendencias observadas en las zonas tropicales, donde grandes superficies de bosque nativo continúan siendo deforestadas, los cambios de uso del suelo en Europa y America del norte van en la dirección contraria. Este efecto ha sido acelerado en parte debido a la puesta en marcha de la Directiva EU 2080/92 de reforestación de tierras agrarias, la cual derivó en una gran superficie de terrenos reforestados en el norte de España entre 1992 y 2006. Estas reforestaciones fueron realizadas mayoritariamente con especies de crecimiento rápido, fundamentalmente *Eucalyptus globulus* Labill., *Eucalyptus nitens* (Deane & Maiden) Maiden y *Pinus radiata* (D. Don), y establecidas en su mayoría sobre praderas.

Los acuerdos internacionales sobre reducción de emisiones de carbono permiten a los países firmantes la compensación de emisiones de CO_2 mediante acumulación en sumideros reconocidos como tal. Sin embargo, hay una elevada incertidumbre asociada a la estimación de la cantidad de carbono secuestrada en los sistemas forestales, principalmente en algunos compartimentos concretos como por ejemplo el suelo mineral. La incertidumbre es aún mayor en sistemas que, tras un cambio de uso, aún no han alcanzado el equilibrio, por lo que el estudio de la evolución del carbono mediante la metodología habitual, suponiendo cambios de stock en el estado de equilibrio, podría acarrear importantes errores en la estimación.

Aunque la estimación del carbono en la biomasa arbórea es relativamente sencilla, la estimación en otros compartimentos, como por ejemplo el suelo, es mucho más complicada y tiene asociada una mayor incertidumbre. Este es el motivo de que la mayoría de los estudios de la dinámica del carbono en el suelo están basados en modelos. Sin embargo, la evaluación empírica es necesaria para estudios más consistentes, y proporciona información valiosa para validación de modelos ecofisiológicos así como para el desarrollo de modelos empíricos. Sin embargo, la medida directa del carbono en el suelo es laboriosa y los resultados son altamente variables, provocando que a menudo no sea posible obtener resultados concluyentes debido a la alta variabilidad. Además, los cambios en el carbono en el suelo son difíciles de estimar debido a que los procesos son relativamente lentos, y a que hay otras variables externas que afectan a su evolución. En este sentido, el diseño experimental mediante cronosecuencias combinadas con parcelas pareadas puede ser útil para convertir diferentas espaciales en diferencias temporales, al mismo tiempo que permiten corregir tendencias locales debido a la alta variabilidad espacial de la variable de interés, permitiendo evaluar la evolución del carbono a escala comarcal.

El carbono en la biomasa arbórea suele estimarse mediante ecuaciones de biomasa, las cuales se les supone una mayor precisión que los factores de expansión de biomasa. Sin embargo, la estimación de la masa seca de los pies tipo que se emplearán en el desarrollo de las ecuaciones de biomasa es posible que presente algún problema mediante las metodologías en uso. Además, el interés creciente en aprovechar fracciones de biomasa de copa para usos energéticos, así como la necesidad de su estimación para estudios nutricionales y ecológicos, lleva a la necesidad de disponer de modelos precisos para la estimación de las fracciones de copa. La inclusión de variables de copa como explicativas en las ecuaciones de biomasa puede llevar al desarrollo de modelos más precisos que los basados únicamente en variables de fuste.

La producción energética de una plantación está habitualmente expresada en términos de volumen o peso seco de madera, mientras que el efecto de substitución de carbono debido al uso bioenergético de los productos forestales suele estar expresado en términos de energía, o toneladas equivalentes de petróleo. La cantidad de energía obtenida por cada unidad de biomasa depende del proceso de transformación, lo que al final lleva a que la estimación de la cantidad de energía se haga en función del volumen en pie o de la biomasa y la aplicación de los factores correspondientes. La existencia de modelos específicos para la estimación de la producción energética en función del estado de desarrollo de la masa puede evitar la concatenación de errores en la estimación, y al mismo tiempo permitir la comparación directa de la producción energética de varias alternativas silvícolas o de diferentes especies.

El primer paso en el estudio la evolución del carbono en el suelo tras un cambio de uso es la evaluación del cambio de stock. Sin embargo, la estabilidad de los compuestos orgánicos del suelo mineral varía tras el cambio de uso. Debido a que las técnicas tradicionales para su estimación son laboriosas y caras, la puesta a punto de técnicas que permitan trabajar con un gran volumen de muestras, y a un coste razonable, será de gran ayuda para estudios de modelización de la estabilidad de la materia orgánica del suelo. Ello cobra mayor importancia cuando se requiere una elevada resolución temporal o cuando se pretende hacer estimaciones a gran escala, para lo cual es necesario analizar una elevada cantidad de muestras.

Cuando se evalúa el efecto de mitigación conjunto del sector forestal, se han de considerar todos los compartimentos en los que el carbono se puede encontrar retenido. Ello incluyen los productos que se generan el los bosques debido a las cortas de madera u otros productos, aunque no está claro aún como estos van a ser tenidos en cuenta en los acuerdos internacionales de reducción de emisiones. Las técnicas más habituales en estos estudios son los modelos mecanísticos, como por ejemplo el CO_2Fix, el cual permite la estimación del efecto de mitigación de varias alternativas silvícolas considerando todos los compartimentos en los que el carbono se puede encontrar retenido en los sistemas forestales. Sin embargo, son escasos los estudios en los que se han evaluado los resultados obtenidos con este tipo de modelos en las condiciones del sur de Europa.

El principal objetivo de esta Tesis Doctoral es la disertación acerca de la modelización y la estimación de la acumulación de carbono en sistemas forestales. Para ello se han considerado

todos los niveles de estimación: árbol individual, rodal y paisaje. El estudio ha centrado en el estudio de un proceso dinámico, como es la aforestación de pastizales en el norte de España. En los distintos capítulos de la presente Tesis se han desarrollado y mejorado herramientas para la estimación de carbono en sistemas forestales. El estudio se ha centrado en plantaciones forestales establecidas sobre antiguos pastizales sobre clima atlántico del sur de Europa, uno de las zonas más productivas de Europa.

El diseño experimental y los métodos empleados en la recolección de datos se resumen en los siguientes puntos, correspondientes a distintos capítulos de la Tesis.

- ✓ Se estableció una red de 120 parcelas pareadas (cada parcela consta de una antigua pradera y plantación forestal establecida sobre la anterior, de diferentes edades), establecidas a modo de cronosecuencia para las tres especies mas habituales en aforestación de terrenos agrícolas en el norte de España. Las parcelas pareadas representaron el uso que originariamente cubría la totalidad de la parcela (pradera), y la plantación forestal establecida en parte de la parcela sobre el anterior uso. En cada cronosecuencia, la densidad de carbono por hectárea fue evaluada en biomasa arbórea, mantillo y suelo mineral hasta 30 cm de profundidad. La evolución del carbono en el suelo mineral y en el mantillo debida a la edad transcurrida desde la aforestación fue evaluada mediante análisis no paramétrico, mientras que el carbono en la biomasa aérea fue evaluado mediante análisis de regresión. El efecto del turno de corta en el stock de carbono para las evolución media de las parcelas medidas también fue evaluado.
- ✓ Se apearon un total de 40 pies de *E. nitens*, los cuales fueron sometidos a análisis destructivo para evaluar su peso seco. Los pies fueron divididos en los siguientes componentes: madera, corteza, ramas gruesas, ramas finas, ramillos, hojas y ramas secas en el fuste. Para evaluar la masa seca de madera se empleó una intensidad de muestreo muy elevada, que permitió evaluar el efecto de ésta en la estimación de la masa seca de madera del árbol tipo cuando se aplica la metodología de *ratio type estimators*. Se evaluaron dos metodologías diferentes: pesado completo del árbol en verde y estimación de la masa seca mediante discos tomados sistemáticamente a lo largo del fuste para determinar la humedad (*CW*), y cubicado completo del fuste y estimación de la masa seca mediante la estimación de la densidad básica (*PW*). Además, se probó el ajuste de algunas variables de copa como predictivas en las ecuaciones de biomasa. La capacidad de las ecuaciones desarrolladas para la estimación de la proporción de las distintas fracciones de biomasa fue evaluada para distintas clases dimensionales.
- ✓ Se desarrollaron modelos estáticos de crecimiento para las dos especies de eucalipto estudiadas, para lo que se midieron 15 parcelas adicionales para *E. globulus* y 36 para *E. nitens*, todas ellas establecidas sobre suelo forestal. Para el desarrollo de los modelos, se tuvieron en cuenta los límites máximos de densidad debido al autoclareo así como otras limitaciones dimensionales derivadas de la posibilidad de cosecha mediante maquinaria convencional. Los modelos permitieron evaluar el efecto de dos densidades iniciales en la

producción energética evaluada mediante varios indicadores, así como la acumulación de carbono en distintos compartimentos para las dos especies de eucalipto consideradas.

- ✓ Varias muestras consideradas como representativas de la evolución media de las parcelas de *E. globulus* y *P. radiata* fueron analizadas mediante calorimetría y análisis térmico, como técnicas recientes para la evaluación del efecto de la aforestación de terrenos agrícolas abandonados sobre la naturaleza de la materia orgánica del suelo y del metabolismo microbiano. Los resultados fueron comparados con los obtenidos mediante una técnica bien contrastada para la evaluación de la naturaleza de la materia orgánica, la resonancia magnética nuclear en estado sólido. La aplicación de las dos técnicas permitió la estimación de la naturaleza de los cambios en la composición de la materia orgánica de los suelos tras el cambio de uso de pastizal a plantación forestal, y puso de manifiesto la capacidad de la calorimetría y el análisis térmico para diferenciar grupos de compuestos de la materia orgánica del suelo.
- ✓ El modelo mecanístico CO_2Fix fue parametrizado con los datos obtenidos en los apartados anteriores y con datos adicionales recopilados del sector forestal en el norte de España respecto al destino industrial de los productos por especie y rendimiento en la transformación. La parametrización del modelo CO_2Fix fue validada para las condiciones descritas en el presente estudio con los resultados obtenidos en las 120 parcelas de los apartados anteriores, donde el carbono en biomasa, mantillo y suelo mineral fue evaluado mediante medición directa. Una vez parametrizado el modelo, se evaluó el efecto de mitigación de carbono en distintos compartimentos para dos alternativas selvícolas y cada una de las especies estudiadas.

Respecto a la evolución del carbono tras el cambio de uso de pradera a plantación forestal (Capítulo II), la acumulación media de carbono en biomasa y suelos para el total de las parcelas de cada especie osciló entre 8.7-14.6 Mg C ha^{-1} $año^{-1}$ (*Eucalyptus nitens* > *Eucalyptus globulus* > *Pinus radiata*), y la contribución del suelo (mantillo + suelo mineral) sobre el total osciló entre 8-18% (*Eucalyptus nitens* > *Pinus radiata*> *Eucalyptus globulus*). El clima templado-húmedo y la textura del suelo franco-arenosa favorecieron las elevadas pérdidas de carbono en el suelo en los horizontes superficiales durante los primeros 10 años tras el cambio de uso. Las pérdidas fueron debidas en parte a los elevados contenidos de materia orgánica en el uso del suelo previo. Las elevadas pérdidas de carbono observadas en las parcelas de *P. radiata* (26% del contenido inicial de carbono, frente al 19,5% de pérdida en las parcelas de ambos eucaliptos) fueron atribuidas a la menor tasa de transferencia de materia orgánica fresca al suelo mineral, debido a una menor tasa de descomposición del mantillo y a una menor incorporación de litter procedente de las raíces de las especies herbáceas. Este último factor fue debido a diferencias en la sombra proyectada por ambos grupos de especies, lo que al final derivó en diferencias microclimáticas bajo cubierta. Las condiciones climáticas favorecieron el rápido crecimiento de la biomasa arbórea y consecuentemente del aporte de matera orgánica al suelo mineral.

Para evaluar los factores que controlan la acumulación o pérdida de carbono en el suelo mineral tras el cambio de uso, se dividió la curva de evolución media de todas las parcelas en dos partes, y se buscaron diferencias entre ambas. Se pudo comprobar que la parte de la curva que suponía unas menores pérdidas de carbono y una recuperación del valor inicial más rápida eran aquellas en las que había un mayor índice de sitio, aunque este efecto no se encontró como significativo. Sin embargo, el contenido inicial de carbono en las parcelas de pradera si que mostró un efecto significativo, de forma que a mayor contenido inicial mayor pérdida y recuperación más tardía. Este efecto fue significativo para los dos eucaliptos pero no para *P. radiata*, posiblemente debido a que para esta especie, todas las parcelas pierden carbono en el suelo en los primeros años tras el cambio de uso independientemente del contenido inicial, debido a un fuerte sombreo sobre el suelo y mortalidad del estrato herbáceo.

Los resultados de las tendencias promedio de evolución del carbono en los distintos compartimentos mediante los análisis no paramétrico y de regresión permitieron evaluar el efecto de la edad de rotación en el stock de carbono y en la tasa de acumulación para las tres especies estudiadas. La capacidad de captura de C de las plantaciones forestales puede ser maximizada mediante el alargamiento de la rotación y la adopción de estrategias adecuadas de gestión para cada especie. Esto es especialmente importante en las plantaciones en las que la alta intensidad de aprovechamiento puede evitar la acumulación de carbono en el suelo a largo plazo.

En el Capítulo III, el muestreo intensivo considerado permitió evaluar el efecto de la intensidad de muestreo y el punto inicial de muestreo en el tronco en la precisión y el sesgo en la estimación de la masa seca de madera en el fuste mediante dos *ratio type estimators* y muestreo sistemático. Para ambas metodologías consideradas (*CW* y *PW*), los residuos se representaron gráficamente frente a la intensidad de muestreo y se establecieron umbrales mínimos de muestreo para mantener el error relativo por debajo del 10%. Los aumentos en el contenido de humedad y densidad básica a lo largo del fuste explican el grave riesgo de sobreestimación de masa seca de madera cuando se aplica muestreo sistemático. De los métodos considerados, el *CW* mostró los mejores resultados para intensidades de muestreo similares para la clase dimensional más grande. Esto es importante porque el método del *PW* se utiliza generalmente para los árboles grandes en los que el pesaje completo del árbol es muy complicado debido a su tamaño.

Los resultados muestran una tendencia clara en todos los pies tipo de los errores cometidos mediante la consideración sistemática de la sección a la altura del tocón como el punto de partida del muestreo. Ello dio lugar a sobreestimaciones en el método de *CW* y subestimaciones en el método del *PW*. En el caso de optar por un muestreo sistemático, sería conveniente aleatorizar la posición de la primera sección muestreada después de establecer la intensidad de muestreo para un error asumido. En el caso del método del *PW*, no se recomienda tomar una única muestra por árbol, sino que la muestra debe ser dividida a lo largo del tallo en un intento de representar la densidad básica media, la cual en la especie estudiada se produjo a una altura relativa de 30-35%.

Las ecuaciones de biomasa fueron ajustadas simultáneamente mediante ajuste ponderado para corregir la presencia de heterocedasticidad. El diámetro normal fue la mejor variable explicativa, y la inclusión de la altura no mejoró la precisión de la estimación para ninguna fracción a excepción de la madera. La inclusión de variables de copa mejoró la capacidad predictiva de las fracciones de copa, aumentando la precisión de la estimación de las ramas gruesas (10,8%), ramillos (19,1%) y hojas (17,3%). La importancia en peso de cada fracción de biomasa siguió el siguiente orden: madera > corteza > ramas gruesas > ramas muertas a lo largo del fuste > hojas > ramas finas > ramillos. Los cambios en los porcentajes de las distintas fracciones sobre el total con el diámetro y la capacidad predictiva de las ecuaciones ajustadas también fueron estudiados.

En el Capítulo IV se desarrollan modelos estáticos a nivel de rodal para la estimación de la producción de biomasa, energía y el secuestro de carbono para primeras rotaciones en plantaciones de *Eucalyptus globulus* y *Eucalyptus nitens* establecidas en el rango habitual de densidades para este tipo de plantaciones en el suroeste de Europa. El volumen, biomasa total aérea, biomasa restos de corta, biomasa de copa, carbono en la biomasa aérea y mantillo, energía en biomasa aérea, energía en los restos de corta y la celulosa extraíble se representaron en forma de isolíneas (considerando la mortalidad) y se representaron frente a la altura dominante.

Las variables anteriores fueron calculadas para dos opciones selvícolas, una destinada a la producción de bioenergía y otra representativa de la selvicultura estándar para las plantaciones de las especies consideradas en el sur de Europa. Los niveles de producción fueron más altos en *Eucalyptus nitens* que en *Eucalyptus globulus* para todas las variables, debido a su mayor crecimiento diametral a similares densidades. El rendimiento total en términos de biomasa fue 13.9-14.6 Mg ha^{-1} $año^{-1}$ para *Eucalyptus globulus* y 20.4-21.5 Mg ha^{-1} $año^{-1}$ para *Eucalyptus nitens*. La energía en la biomasa aérea varió entre 233-245 GJ ha^{-1} $año^{-1}$ para *Eucalyptus globulus* y 345-364 GJ ha^{-1} $año^{-1}$ para *Eucalyptus nitens*, la tasa de acumulación de carbono en la biomasa aérea y mantillo fue 6.9-7.2 Mg ha^{-1} $año^{-1}$ para *Eucalyptus globulus* y 12.7-13.5 Mg ha^{-1} $año^{-1}$ para *Eucalyptus nitens*, y la celulosa extraíble fue 5,7-5,9 Mg ha^{-1} $año^{-1}$ para *Eucalyptus globulus* y 9,0-10,1 Mg ha^{-1} $año^{-1}$ para *Eucalyptus nitens*.

Las rotaciones simuladas fueron más largas que las habitualmente consideradas en las plantaciones energéticas leñosas tradicionales. Esto tiene algunos efectos positivos, como la reducción del ratio madera:corteza y el mayor tamaño promedio de los árboles. Se observó que un incremento del 50% en la densidad inicial con respecto a la densidad estándar resultó en incrementos marginales en la biomasa y celulosa extraíble por hectárea. Los diagramas de manejo de la densidad constituyen una herramienta útil para la gestión de las plantaciones de eucalipto con un objetivo múltiple (pulpa o la producción de madera sólida, sustitución de combustibles fósiles o el secuestro de carbono).

En el Capítulo V se describe la aplicación de la calorimetría diferencial de barrido (DSC) y análisis térmico como nuevas técnicas para dilucidar cómo la aforestación afecta la naturaleza de la materia orgánica del suelo y el metabolismo microbiano del suelo. Las técnicas se han aplicado

para estudiar la dinámica de la materia orgánica edáfica en terrenos aforestados con *Pinus radiata* y *Eucalyptus globulus* establecidas sobre pastizales en una región templada húmeda. Los resultados del análisis térmico y calorimetría se compararon con los obtenidos por resonancia magnética nuclear en estado sólido (NMR), técnica bien contrastada para examinar los cambios en la estabilidad química de la materia orgánica.

La aplicación de DSC y NMR reveló que la materia orgánica edáfica en los primeros años tras el cambio de uso estaba compuesta por hidratos de carbono, grupos carbonil/carboxil, componentes alifáticos y aromáticos. Tras el cambio de uso del suelo, hubo una pérdida de materia orgánica en todos los compuestos descritos. Esta pérdida de materia orgánica fue detectada por el ratio calorespirométrico del metabolismo basal del suelo, junto con la biomasa activa y el cociente metabólico. Estos índices demostraron ser sensibles a los parámetros que proporcionan información sobre los cambios en los patrones de metabolismo microbiano en respuesta a cambios en la naturaleza y el estado redox de los sustratos de carbono, lo que demuestra la degradación de fracciones de materia orgánica aromática y alifática. Las técnicas fueron capaces de distinguir las diferencias en la dinámica de materia orgánica del suelo para las dos especies, atribuible a diferencias en el desarrollo de vegetación del sotobosque y la composición del mantillo.

En el Capítulo VI se llevó a cabo un amplio estudio del efecto de mitigación de carbono para las especies estudiadas bajo las condiciones particulares de la del sector forestal en el sur de Europa, teniendo en cuenta el efecto sumidero de C en los productos forestales, suelo, biomasa viva y efecto sustitutivo del uso bioenergético. El modelo CO_2Fix fue parametrizado mediante los modelos locales de crecimiento, turnover rates de hojas, ramas y raíces estimados mediante modelos locales y parámetros específicos de la región para los productos forestales. La validación se llevó a cabo para los módulos de biomasa y suelo con datos reales de 120 parcelas, considerando las características climáticas específicas de cada parcela. Los resultados mostraron un fuerte sesgo en la estimación del carbono en el suelo, que fue atribuido a una sobreestimación de las tasas de descomposición. También se observó una ligera subestimación cuando modelos específicos para zonas forestales fueron aplicados para la obtención de las tablas de producción para plantaciones forestales establecidas sobre terrenos agrícolas.

El modelo Yasso se mostró fuertemente robusto respecto a los turnover rates de ramas, hojas y raíces. Dentro de las alternativas selvícolas evaluadas, la destinada a la producción de madera de trituración derivó en mayores stocks tras la consecución del de equilibrio en biomasa y productos, así como en el efecto sustitución debido al uso bioenergético que la alternativa de producción de madera aserrada. Sin embargo, la alternativa de madera aserrada fue más efectiva en cuanto al stock de C en el suelo tras la consecución del estado de equilibrio. El índice del sitio tuvo un efecto importante para todas las especies, alternativas y compartimientos, de forma que a mayor índice de sitio mayor efecto de mitigación. El aprovechamiento de restos de corta para uso energético dio lugar a una ligera disminución carbono en el suelo en el estado de equilibrio, pero aumentó significativamente el efecto de mitigación debido al uso bioenergético.

Chapter I

General introduction and objectives

1. General introduction and objectives

1.1. Climate change and the forest sector

1.1.1. Climate change, a global problem

Almost the entire scientific community now accepts the evidence that the climate is changing. This effect has been reported in relation to the trend in annual temperatures in the last 30 years (Trenberth & Josey, 2007), and on a longer timescale in the last century (Jones & Moberg, 2003), and in the last 1000 years (Jones *et al.*, 2001). Although there have been examples of natural climate oscillations throughout the Earth's history, the current effect is attributed to anthropogenic activity (IPPC, 2001; Grace, 2004). The two main reasons for climate change are increasing emissions of greenhouse gases (GHGs) and aerosols, and land use change, both of which cause changes in atmospheric temperature (Houghton *et al.*, 1990) as a result of the so called greenhouse effect (GE).

The main GHGs are water vapour, CO_2, CH_4 and N_2O. Emissions of GHGs used to be expressed in terms of CO_2-equivalent because CO_2 alone contributes more than 30% to the total greenhouse effect in the atmosphere, 56% of the anthropogenic radioactive forcing (Hansen & Lacis, 1990), more than 82% to total emissions at European level (IPPC, 2007), and also because it can be captured through biological processes. Plants, as autotrophic organisms, transform inorganic compounds into organic tissues, consuming CO_2 in the process. Releases via respiration and sequestration by biomass growth were in equilibrium until the beginning of the industrial age, when huge amounts of the system carbon (C) was released as a result of increased burning of fossil fuels carried out in response to increased energy demands (Foley *et al.*, 2005).

Some scientists think that climate change is irreversible (Solomon *et al.*, 2009). Nonetheless, even greater increases in the average annual air temperature will occur unless mitigation policies are adopted (Meehl *et al.*, 2007). The development of adaptation strategies and evaluation of the predictable impacts on each sector as regards the new situation are two of the most active research lines at the moment (Campioli *et al.*, 2009). There are several ways of reducing CO_2 emissions by the amounts required by international agreements, but these can be summarized in two sets of actions: increasing the C sink, and reducing C emissions.

Worldwide, GHGs emissions are mainly related to energy use (63%), predominated by electricity power plants, industry and transport (Fig. 1.1). Reduction in GHGs emissions therefore

requires a change in energy supply to alternative renewable energy sources. Although there are various CO_2-free alternatives for power and industry energy supplies (i.e. nuclear, wind, hydraulic), as regards transport, the use of biofuels has received extensive attention because at present there are no other CO_2-free alternatives available with the technology in use (Fulton *et al.*, 2004). Some authors indicate that for a significant mitigation of fossil fuel emissions, very large areas of cropland would be required to produce sufficient amounts of biofuel for transport needs (Righelato & Spracklen, 2007), which may lead to displacement of agricultural production and cause additional land-use change, finally leading to net increases in GHGs emissions (Searchinger *et al.*, 2008; Melillo *et al.*, 2009). In this sense, only conversion of woody biomass may be compatible with retention of forest carbon stocks (Kirschbaum, 2003; Tilman *et al.*, 2006; Righelato & Spracklen, 2007).

In Spain, 80% of GHGs emissions are derived from the energy sector, and renewable energies have been considered as an important alternative for reducing emissions, with biomass having a neutral emission effect. The 2005-2010 Renewable Energy Plan (PER) in Spain aimed at a 12% contribution of renewable resources for primary energy consumption by 2010. However, the current situation clearly shows that biomass is the least well developed among the renewable energies in Spain. The share of forest biomass is difficult to calculate, although it is clear that recent initiatives for new power plants are mainly linked to the use of forest and agricultural industry residues, and that forest energy crops (short rotation coppice) would be important within the group of energy crops. One of the key elements for the previous points is Royal Decree RD 661/2007, which establishes the grant system for alternative energy production. The Decree allocates a large portion of the incentives to power plants that utilise biomass from energy crops.

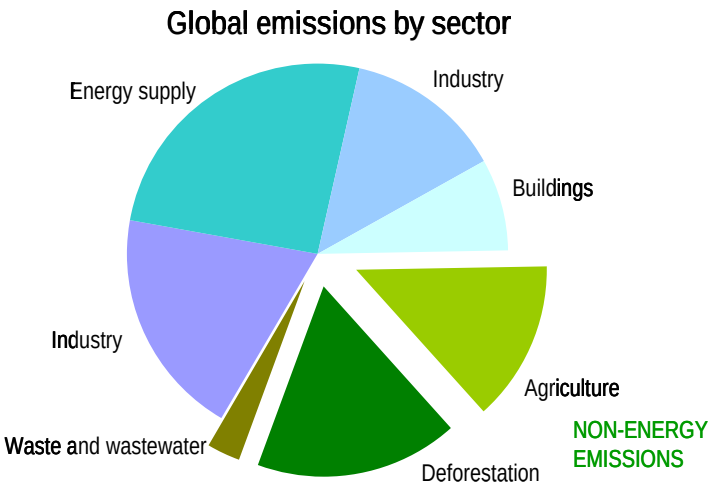

Figure 1.1. Global greenhouse gases emissions by economic sector. Source: IPPC (2007).

Increase of terrestrial sinks also lead to decreases in the concentration of atmospheric CO_2, although the mitigating effect of this option is limited. Non energy emissions account for up to 37% of global emissions (Fig. 1.1), and a change in land use to others with more favourable C balance may partly mitigate the greenhouse effect. Moreover, alternative management schemes for current

land uses may provide different C balances, and must therefore be investigated (Malhi *et al.*, 2002).

1.1.2. International agreements regarding reduction of GHG emissions

All administrations and policymakers now acknowledge climate change as one of the most important current environmental problems, and several attempts have been made to obtain international agreement for reducing CO_2 emissions. Inclusion of carbon sinks in the global carbon budget was discussed at the United Nations Framework Convention on Climate Change (UNFCCC) celebrated in Rio de Janeiro (1992). However, it was at the Kyoto meeting (UNFCCC, 1997) that the UNFCCC limited GHGs emissions, taking as a baseline for accounting the emissions levels in 1990; it was also agreed that emissions could be compensated through sinks. This document recognizes two main alternatives for emissions compensation in the Land Use, Land Use Change and Forestry (LULUCF) sector for industrialized countries, depending on whether the actions took place within their own territory (Art. 3.3, 3.4 and 6) or in other non-industrialized countries (Art. 12). For actions within their own border, the Kyoto Protocol (KP) allows countries included in Annex I the following activities to meet the requirements of the KP:

- ✓ Article 3.3, to use *"net changes in greenhouse gas emissions by sources and removals by sinks resulting from direct human-induced land-use change and forestry activities, limited to afforestation, reforestation and deforestation* (ARD) *since 1990"* (UNFCCC, 1997).
- ✓ Article 3.4: *"forest management* (FM)*, cropland management* (CP)*, grazing land management* (GM) *and revegetation* (RV) *are eligible land-use, land-use change and forestry activities"* (UNFCCC, 2001).
- ✓ Article 6: *"transfer to, or acquire from, any other such party reduction units resulting from projects aimed at reducing anthropogenic emissions by sources or enhancing anthropogenic removals by sinks of greenhouse gases in any sector of the economy, provided that (…) any such project provides a reduction in emissions by sources, or an enhancement of removals by sinks, that is additional to any that would otherwise occur"* (UNFCCC, 1997), which is known as joint implementation (JI).
- ✓ Article 12, Annex I countries are allowed to acquire from non-Annex I countries certified Emissions Reduction Units (ERUs), through the so called Clean Development Mechanism (CDM), which provides that C removal projects are *"additional to any that would occur in the absence of the certified project activity"* (UNFCCC, 1997), and is limited to afforestation and reforestation activities for the first commitment period (UNFCCC, 2001).

JI and CDM are required to consider a baseline for the certified emission reductions, in other words the reference C level with respect to gains or losses must be estimated, and for agricultural activities (CM, GM, and RV), net-net accounting is required, which consists of considering *"net emissions or removals over the commitment period less net removals in the base year, times five"*.

In subsequent meetings (UNFCCC, 2002; UNFCCC, 2003) the instructions for GHGs inventories in the LULUCF sector were defined. This arrangement defines the methodology, the default values and the pools that must be considered in forest GHGs inventories, as follows: live above and belowground biomass, litter, dead trees and soil organic matter. Carbon in forest products cannot therefore be considered in carbon accounting calculations (UNFCCC, 2002).

The results of the last meetings of the United Nations Climate Change Conferences (COP) celebrated in Copenhagen (COP15, 2009) and Cancun (COP16, 2010) are ambitious in regard to GHGs reduction and deferred the force period of KP. However, the current economic crisis, the generalized dependency of fossil fuel energy, and the permissiveness with developing countries with high emissions rates, makes compliance of the international agreements on emission reductions difficult. GHGs emissions increased by a record amount in 2010, to the highest carbon output in history (30.6 Tg), according to the International Energy Agency, putting hopes of holding global warming at safe levels all but out of reach.

Total emissions increased between 1990 and 2008 in Spain by on average 47.8%; inclusion of LULUCF supposes a reduction of 15.1 points as regards the latter figure (UNFCCC, 2010). For the period 2008-2012, Spain is committed to increasing emissions by 15% relative to the base year (1990), which is included in the emission allowance trading scheme within the EU, i.e. global reductions in emissions of 8%. Nonetheless, the previsions of the government (II Assignation Plan) consider an increase of 37% the most likely estimation.

The Spanish Climate Change and Clean Energy Strategy (EECCEL) considers that the increasing trend of GHG emissions in the 1990-2008 period corresponded to rapid, sustained economic growth, and to an increase in population in recent years. The effort made by Spain in matters of Energy Saving and Efficiency was insufficient, but the report also shows that the per capita emissions reached an average of approximately EU-15. The EECCEL states that the target established by the Government for the five-year period 2008-2012 is that Spain's totals do not surpass a 37% increase relative to the emissions in the base year. This represents a difference of 22 percentage points with respect to +15%, 2% of which must be obtained by means of sinks, and the remainder (20%) by means of flexible mechanisms (acquisition of carbon credits). In order to reach the said objective of +37%, the National Allocation Plan (NAP) 2008-2012 requires additional measures to obtain reductions of 27.1 Mt of CO_2 eq. A Plan of Urgent Measures considers reductions of 12.091 Mt CO_2 eq yr^{-1}, so that additional measures are still necessary to provide reductions of 15.033 Mt CO_2 eq yr^{-1}.

1.1.3. The role of forests and the forest-based sector in climate change mitigation

As previously reported, the main research lines regarding climate change and the forest sector are impacts, mitigation and adaptation (Campioli *et al.*, 2009). Mitigation is the action taken to reduce the atmospheric concentration of GHGs in order to prevent dangerous climate change (IPPC, 2007). However, as GHGs mitigation alone is not sufficient to prevent climate change, we

must be prepared for the possible impacts of increased temperatures and changes in precipitation regimes on forests.

Several impacts are currently under study; most of these are related to change in forest productivity, abiotic and biotic disturbances, and species migration and extinction (Thuiller, 2003; Thomas *et al.*, 2004; Battisti *et al.*, 2005; Blennow & Olofsson, 2008; Lindner *et al.*, 2010; McMahon *et al.*, 2010). The susceptibility of forests to these impacts depends on the inherent adaptive capacity of trees and forest ecosystems (Hamrick, 2004; Thuiller *et al.*, 2005) as well as on the intensity and direction of climatic variables. Adaptation is adjustment in natural or human systems in response to actual or expected climatic stimuli or their effects (IPPC, 2007).

There are several ways in which forests and the forest-based sector can contribute to mitigating the greenhouse effect. In simplified terms, the forest-based sector can be seen to be formed by the forest ecosystem, forest products, and energy from the forests. Canadell & Raupach (2008) indicated four forest management strategies for mitigating GHGs emissions: (i) increasing the forest area through reforestation, (ii) increasing the C density per area, (iii) increasing forest product use and fossil fuel substitution through bioenergy, and (iv) reducing deforestation and degradation. Together these proposals can be summarized as increasing C stock in all compartments in which forests can contribute (biomass, soil, products), and generating a stream of avoided emissions through bioenergy use.

Decreasing the forest area is one of the most important factors as regard release of CO_2, N_2O and CH_4 to the atmosphere (Shukla *et al.*, 1990; Malhi *et al.*, 2008). This is still occurring in the tropics (Canadell & Raupach, 2008), whereas the forest area is generally increasing in temperate and boreal regions (FAO, 2005). One of the most evident ways of mitigating climate change through the forest sector is therefore to increase forest area or maintain the area by slowing down deforestation, although this is difficult because forest land in developing countries is being transformed to agriculture land to meet human feed requirements. In this context, the area covered by forest plantations is expected to increase by 16-32% by 2030 relative to the level in 2005 (estimated at 261 M ha, Carle & Holmgren, 2009). According to these authors, the highest absolute increase will take place in Asia, whereas the highest relative increase will occur in Southern Europe.

Other ways of increasing the C stock are to optimize management schemes regarding C sequestration (Liski *et al.*, 2001; Liski *et al.*, 2002; Kaipainen *et al.*, 2004; Canadell & Raupach, 2008). In traditional forestry, management schemes consider wood supply or economic criteria, and other types of management schemes that favour better C balance may not be compatible with traditional management practices (Lindner & Karjalainen, 2007). Additional research is therefore needed to evaluate the effect of actual and potential management schemes on the different C pools considered in the international agreements (UNFCCC, 2002).

Finally, the last way in which the forest sector can mitigate climate change is known as the substitution effect. This involves the so-called avoided emissions, derived from consumption of harvested wood products (HWP), rather than other products that require consumption of fossil

fuels during their fabrication. HWP are not considered in global C budgets unless a country can show that its long term existing stocks are increasing (UNFCCC, 2002), although the 26th session of the Subsidiary Body for Scientific and Technological Advice (UNFCCC/SBSTA/2007/4, paragraphs 59-61) had invited parties in a position to do so, to report voluntarily on wood products in their national inventories. This is because the strong effect of HWP on the global carbon cycle due to the avoided emissions from sources that have stored carbon for thousands of years. Therefore, even when the emissions from HWP are immediate (in the case of bioenergy), or when the wood product would have a longer lifespan (furniture, paper, etc.), the avoided emissions to obtain the same amount of energy or to manufacture the alternative product are derived from a pool that has been stored for thousands of years.

Keeping forests at an intense stage of growth may help maximize the supply of forest products, and therefore maximize the mitigating effect, although this does not mean that changes from mature virgin forests to plantations should be envisaged. Some studies have shown the ability of old growth forest to capture C (Carey *et al.*, 2001; Pregitzer & Euskirchen, 2004; Zhou *et al.*, 2006; Luyssaert *et al.*, 2008), and other studies have reported that change from old-growth forest to young fast growing forest does not result in a positive net C balance, even if wood products are considered (Harmon *et al.*, 1990). Holding forests at fast growing stages implies intense management, which may finally lead to losses of soil organic carbon (SOC), although the balance is usually towards net sink effects.

The fight against climate change may produce restrictions, but also opportunities: mitigation can reduce our external dependence on fossil fuels and alleviate environmental problems, such as urban contamination. Land use planning can also be improved and clean transport systems promoted.

1.2. The forestry sector in northern Spain and climate change mitigation

1.2.1. Forestry, land-use change and carbon sequestration in Spain

The rural landscape underwent a change during the last century, motivated by the change in economic activity. At the beginning of the 20th century, most of the primary economic sector was based on agriculture and livestock. The normal development and modernization of the primary sector were held up by the civil war and the posterior recovery period. From the mid-1950s, depopulation of rural areas led to huge abandonment of land (Lasanta, 1996; Lavorel *et al.*, 1998); agriculture production was concentrated in the most productive areas, and less productive land was abandoned or transformed into forest.

During this time the Spanish Government initiated an extensive afforestation program across the country, which led to in a huge areas of land being afforested. In the period 1940-1973, the total area afforested in Spain was 2306200 ha (Pemán García *et al.*, 2009), and 48% of this corresponded to afforestation of non forest land. The main objectives of this policy were to increase the forest cover and to reduce rural unemployment. The most commonly used species in this extensive afforestation program were coniferous species, because the land available was the least productive land (Pemán García *et al.*, 2009). As regards land property, most of this afforestation involved public land, although some involved collectively owned land.

More recently (in 1992), the European Union implemented a policy to afforest former agricultural land (EEC 2080/92), which led to afforestation of approximately 160000 ha of land in northern Spain (MAPA, 2006). Although the main aim of this policy was to reduce agricultural surpluses, the effect on C stock is still under study. Livestock predominates in the agricultural sector in the north of Spain, the most frequent land use change was therefore from pasture to forest land. Pasture land was usually managed in rotation with rape-seed, with increasing importance of the pasture as abandonment became stronger.

Finally, almost 3.0 M ha of land were reforested in Spain in the last century, of which at least 1.4 M ha was non forest land, predominantly agricultural land (Pemán García *et al.*, 2009). In 2005 the total area covered by planted forests in Spain was 1.4 M ha (Del Lungo, 2009). Because of the peculiarity of this land use change, research is required to evaluate the effect of this policy on the global C sink.

The main objectives of the Spanish forest-based sector are to increase the capacity of CO_2 sequestration from the atmosphere by wood stocks, and to comply with the goal of compensating 2% of the base year emissions by LULUCF. The role of Spanish forest in climate change mitigation has been investigated in specific studies (Bravo, 2007; Pardos, 2010), which reported a value of 670 M t CO_2 for carbon stored in the aboveground biomass of trees, whereas Gracia *et al.,* (2005) reported a higher value of 2050 M t CO_2 for a total carbon in forests, with an annual net increase in sequestration equivalent to 40 M t. The balance between National Forest Inventories IFN2 (1986-1996) and IFN3 (1997-2006) has been studied in detail in some areas (Bravo, 2007) and the values for increased C (new forests, growth of existing trees, ingrowth) and decreased C (felling, natural mortality) were found to lead to a net increase of 54.6%.

This same source of information (IFN) shows that in all regions Spanish forests have acted as C sinks during the 20th century, with a net increase in the sink effect in the period 1990-1998 relative to the period 1974-1987 (Rodríguez-Murillo, 1999). Between the last two inventories, the total amount of C accumulated in the aboveground biomass ranged between 4.5 Mg C ha^{-1} for Galicia (2.0 in the previous period) and 1.1 Mg C ha^{-1} in Murcia (0.27 in the previous period). This change is attributed to the reforestations carried out in Spain in the 1940s, and to more recent changes in land use, mainly in agricultural land, which led to an increase in the forest area as well as in carbon density (Rodríguez-Murillo, 1999). However, in these estimations it is assumed that

afforestion on former agricultural land follows the same dynamics as secondary successions or reforestations, although this is still under study.

1.2.2. The tree species studied

The contribution of forest plantations to the global wood supply is unquestionable. While forest plantations represented less than 7% of global forest area worldwide in 2005 (FAO, 2005), in the same year they satisfied two-thirds of global industrial roundwood needs (Evans *et al.*, 2009). In the north of Spain, forest plantations also predominate in the forest industry supply, and fast growing tree species are the most commonly used in afforestation (Álvarez, 2004). Pines and eucalypts are the most frequently used species in forest plantations, and in plantations on former agricultural land, the most commonly used tree species in north-western are *Eucalyptus globulus* (Labill), *Eucalyptus nitens* (Dean & Maiden) Maiden and *Pinus radiata* (D.Don).

Eucalyptus globulus (Labill)

Eucalyptus globulus is native to south-eastern Australia (Victoria) and the south coast of Tasmania, where it grows in the lowest zones of the island (0-550 m); the average precipitation in the zone ranges between 600 and 1500 mm, in a temperate-type clime. This species was first introduced in Europe from Australia in 1863 (Ruiz de la Torre *et al.*, 1979), and nowadays is distributed along the entire coast of the Atlantic Iberian Peninsula. Frost tolerance is the principal constraint for planting at elevations higher than 400-500 m. The range of rainfall in the areas where it has been introduced is very broad, and the average value for the North and Nothwestern Iberian Peninsula is higher than 1500 mm, which explains the good yields. One of the main problems of this species as regards forest growth is disease attack, which causes reduced yield through defoliation at the young stage (*Mycosphaerella* spp. (Otero *et al.*, 2007)), or at the adult stage (*Gonipterus scutellatus* (Mansilla Vázquez, 1992)). Although several resistant clones of *E. globulus* *Mycosphaerella* are available nowadays, infection of the adult stages by *Gonipterus* is more difficult to manage.

Initially the species was widely used for mining timber and a range of other uses. Nowadays, eucalyptus timber is mainly used to produce bleached pulp, although production of energy crops is also a promising management goal, because of the high proportion of cellulose in the timber (which can lead to high production of bio-ethanol), and its resprouting ability. Other potential uses under study are as high quality sawn-wood (Nutto & Touza Vázquez, 2004), and also structural wood (Guaita & Eiras, 2007). The so-called chip industry covers the use of eucalypt timber for pulp production, which is the main use for this species, because of its favourable specific consumption relative to other species (Cotterill & Macrae, 1997). Management of stands for this purposes starts with plantation densities between 1300 and 1400 stems ha^{-1}, and thinning is not carried out. After the first rotation (13-16 years), several coppice rotations are expected until new plantations are

established. In each coppice rotation, sprout selection is required to maintain the sprout density constant.

Eucalyptus nitens (Dean & Maiden) Maiden

Eucalyptus nitens is native to the south-east of Australia, where it is discontinuously present in the states of New South Wales and Victoria. This species grows naturally at altitudes between 600 and 1600 m in the temperate zone, with precipitations of between 750 and 1750 mm per year. Mountain blue gum tolerates absolute minimum temperatures of -10ºC in north-western Spain (González-Río *et al.*, 1997), and of 21-23ºC in the hottest month in its region of origin (Boland *et al.*, 1980). *Eucalyptus nitens* is one of the most widely used plantation species worldwide in temperate cold regions, and covered more than 340000 ha in 2004 (INFOR, 2004). Expansion of this species in northern Spain began recently; research trials were established around 1950, and commercial plantations began in 1992 in north-western Spain. The reason for the success was the possibility of expansion in areas that were too cold for *E. globulus*, in addition to the high yield, pest resistance, and the relatively good wood properties of the species.

In contrast to the trend observed for the species in Spain, the main destination for *E. nitens* wood worldwide is the pulp industry (INFOR, 2004). This is partly because of the good mechanical properties of *E. nitens* pulp (Paz, 1999), but also because of its high yield. Nevertheless, the specific consumption of *E. nitens* is higher than that of *E. globulus* (Cotterill & Macrae, 1997), and there are some problems related to pitch, which cause pulp stain. Another interesting property of *E. nitens* is the calorific power of its waste and wood, which makes it suitable for energetic purposes (Pérez *et al.*, 2008).

The most common management scheme for *E. nitens* in northern Spain is planting at densities of 1300-1400 stem ha^{-1}, with no thinning. No coppice regimes are applied in north-western Spain, because the most common provenance used in reforestation in the region (MacAlister), has little resprouting ability (Sims *et al.*, 1999b; Sims *et al.*, 2001; Little & Gardner, 2003). The possibility of coppicing of other provenances in northern Spain is still under study. There is some interest in producing sawn-wood with this species, and thinning and pruning schemes are therefore under study. Another current important line of research is energetic plantations, although the species does not grow particularly well at high densities (Sims *et al.*, 1999a; Sims *et al.*, 2001).

Pinus radiata (D.Don)

Pinus radiata is native to a small area on the west coast of North America (Swanton, Monterrey and Cambria in California, and Guadalupe and Cedros in Mexico), but is one of the most commonly used species in afforestation throughout the world, and also the most commonly used exotic conifer for reforestation in the world (Lavery, 1986) and in Spain. The species was introduced in Spain in about 1850, and henceforth its cover increased throughout temperate-climate Spain (Galicia, Asturias, Cantabria and the Basque Country). The success of expansion was due to its fast growth in temperate-climate regions, the wood quality and the plasticity.

The main destination for *P. radiata* wood is as sawn-wood, but also as woodchip for the chipboard industry. Management schemes for this species in the region depend on the wood destination. For the sawn-wood industry, management consists of planting at 1200-1400 stems ha^{-1}, two thinnings, and a third in cases where high value products are the production objective and site quality is large enough. Products from the first thinning are usually used in the chipboard industry or for bioenergy, and only a few trees (400 stems ha^{-1}) remain standing at the end of the rotation, which in this case is 35-40 years. Two prunings are required to ensure wood quality at the end of the rotation. On the other hand, management for woodchip for use in the chipboard industry starts with higher densities, and the rotations are shorter than for sawn-wood.

1.3. Management tools and methods for carbon inventory and evaluation of mitigation effects in forests

There are several ways of monitoring carbon stocks and the mitigation effect in forest ecosystems, but all can be classified in two main groups: (i) methods based on measuring the system C fluxes (FA), and (ii) methods based on measuring changes in C stock (SA) (Ney *et al.*, 2002; Houghton, 2003; Lindner & Karjalainen, 2007; Dias *et al.*, in press). The main differences between the different ways of estimating the stock and the mitigation effects are that C in the FA approach is evaluated from a dynamic point of view (exchange rate), whereas C in the SA methodology is evaluated from the static point of view (stock). With the first approach attention is focused on the specific process under study, more importance is given to the exchange of C in the system or between two pools in the same system (i.e. soil and living biomass, total system and the atmosphere, etc.). In the second approach, more importance is given to the final result of the physiological process, which in the end is the accumulation of C in the system. Since the FA approach is sensitive to short-term changes in the environmental conditions, erroneous conclusions could be reached from this method unless a representative temporal time series is measured. Moreover, the initial value must be given for assessment of the final stock, and the SA methodology must be applied anyway. The SA approach is therefore the most commonly used method for investigating changes in forest systems and for estimating mitigation effect. However, there is still some uncertainty as regards certain aspects of this methodology, as detailed below. Other methods are used almost exclusively for estimating the mitigation effect associated with harvesting and wood products, these are the methodologies based on measuring production (PA) (Brown *et al.*, 1999; Dias *et al.*, in press).

Depending on the methodology, all approaches may be classified as direct measurement or estimation through previously developed models. Although direct measurement is the most reliable way of estimating the C stock in forest system, this method is time consuming and expensive when an adequate replication is applied. A modelling approach is therefore usually used to determine the

stock or fluxes of C in forest systems. However, empirical determination is required for development and validation both empirical and eco-physiological models. The aims of all modelling approaches is to predict the C from variables that are easy to measure and well correlated with the dependent variable under consideration. This implies that direct measurement of independent variables is required, which is supposed to be easier than direct measurement of the dependent variables. Models can also be useful for assessing the expected future state of forest ecosystems, and the effect of several management strategies related to the variable of interest, although not all models enable this type of study to be carried out. Moreover, there are several scales of estimation in forest systems: tree level, stand level and landscape level, which have implications regarding the degree of uncertainty (Weiss *et al.*, 2000).

Two main approaches can be distinguished in modelling: empirical and eco-physiological modelling. The first is based on empirical relationships between the variable of interest and the predictive variables. These models are like a "black box" in which the processes that enable a value for the ecosystem response to be reached is not important, but the aim is to estimate as accurately as possible the dependent variable from the independent variables. These statistically-based models are easy to develop, and feed and execution times are shorter (Keenan *et al.*, 2008). Moreover, these models require few parameters and have been proposed by the IPPC (2006) for the lower territory level (Tier 2). These models assume that there are no other external variables that affect such an empirical relation, and therefore the validity of empirical models is restricted by the estimation level at which it was constructed and even by unchanged environmental conditions. Therefore, the lack of ability to explore new scenarios and conditions outside of those on which they were built and tested is a major constraint (Keenan *et al.*, 2008). One possible way of correcting this problem is to include environmental variables (i.e. climate) in the empirical relationships (hybrid models).

On the other hand, eco-physiological models focus on describing the physiological process that the modelled phenomena undergo, taking into account all environmental variables related to the specific processes. These models include mathematical descriptions of all processes that govern the system, and describe all of them separately and how they dynamically interact with other processes (Keenan *et al.*, 2008). These models are more complicated and difficult to develop and parameterize because of the large number of parameters needed, but they have some advantages with respect to empirical models: (i) they allow study of the effect of each variable or interactions among variables on the final process, (ii) they are more widely applicable than models that are designed to fit data, and (iii) they enable estimation of the changes in the dependent variable in conditions of climate change. One of the main constraints is associated with the large amount of data required for parameterization, which are not always available. This lack of data sometimes leads to assumptions and approximations in the parameterization (Keenan *et al.*, 2008), which finally may make the models less applicable in the practice than empirical models. However, there are no pure eco-physiological models as some of the processes are described by empirical relationships (Landsberg & Sands, 2011).

1.3.1. Methods based on C flux measurement

The flux measurement approach provides information about the net exchange of carbon in the system. It involves direct measurement of the exchanges of CO_2, water vapour, and energy between terrestrial ecosystems and the atmosphere. The eddy covariance method is increasingly recognised as the standard technique for producing continuous data on the fluxes of these parameters (Landsberg & Sands, 2011), mainly because of its high temporal resolution. This method relies on the fact that relatively coherent, rotating parcels of air characterised by properties such as momentum, temperature, CO_2 and water vapour concentrations, are vertically mixed by turbulence so that their properties are transferred between levels (Landsberg & Sands, 2011). Three dimensional wind measurements are required to decompose the vertical and horizontal component, which finally enables estimation of the exchange variable of interest with the atmosphere after a relatively complex mathematical procedure.

Although more than 400 stations worldwide are providing real information about CO_2 flux exchanges under the umbrella of the FLUXNET program (Baldocchi *et al.*, 2001; Baldocchi, 2008), stand level measurements are time consuming and difficult to obtain in practice because high accuracy and fast-response sensors are required. On the other hand, this method enables estimation of the global exchange of CO_2 from earth ecosystems to the atmosphere, as well as comparison of the flux between different biomes worldwide, and is sensitive to changes in net flux, which may be due to climate change. Changes in CO_2 flux for different land uses can only be evaluated by this method from a static point of view, looking at the observed exchanges in the equilibrium state for all land uses considered, unless micrometeorological tower stations have been installed prior to land use change. Moreover, this method does not distinguish between soil and plant exchange, for which additional measurements or modelling are needed.

Information provided by this method is valuable for understanding and describing the processes of C exchange among the several pools into which forest systems can be divided. However, one of the main constraints of this technique is the evaluation at the small scales at which some of the physiological processes occur, i.e. the exchange of CO_2 between aliphatic organic matter in the soil and the atmosphere. Exchanges between very lowly or highly dispersed pools cannot be estimated by this method because of the size of sensors and sensibility required. Fluxes between some compartments are therefore usually evaluated by the stock change methodology.

Although the techniques traditionally used at landscape level for flux measurements are based on fixed or mobile measurement stations, other techniques are currently being investigated. Several attempts have been made to estimate CO_2 emissions directly by remote sensing techniques, although the reliability of this methodology is still poor (Bateson *et al.*, 2008). Although development of these techniques may provide a simple way of determining the net global exchange at stand or landscape level, thus enabling comparison of different types of land use, regions and management practices, more detailed studies with high accurate measuring stations

are irreplaceable when detailed data are required to feed or develop empirical or mechanistic models. Some of the most commonly used models for soil organic carbon (SOC) estimation may be considered to be based on C fluxes, since exchange among the different compounds in the soil is estimated dynamically from the predicted fluxes among compartments. Most of these are eco-physiological models, in which the complexity of the process described cannot be estimated correctly by methods based on measurement of changes in C stock.

1.3.2. Methods based on measurement of changes in C stock

This approach involves evaluating the C stock in the process under study, and the gain or loss is obtained by difference. The approach can be applied to all compartments considered by the UNFCCC (2002), and enables evaluation of the difference at different times within the same land use, or between different land uses. When ecosystems are in steady state, another way of estimating the C stock change is through evaluation of changes in the area of interest, as regards the C density in the previous land use. Like for the last approach, the SA methodology can be carried out by direct measurement or by modelling.

One of the main problems with this methodology is in describing non static systems, e.g. in which a successional change or a human-induced land-use change occurs in a forest stand. In such cases, the temporal resolution for describing this type of processes implies that very frequent remeasurements of the same site are required. Although this method is widely acceptable, it is time consuming, there is a long delay in obtaining results, and estimates may be biased because of changes in the external variables (i.e. climate). Alternative methods include the combined use of chronosequences and paired plots, which enables geographic differences to be translated into into temporal differences, whereas the use of paired plots enables local tendencies to be corrected. The combined use of chronosequcences and paired plots may enable estimation of changes in the C stock in the compartments of interest, after correcting the local tendencies.

The SA method is also known as an inventory based approach when evaluating the biomass C stock, because it is usually based on traditional forest inventory remeasurement techniques, in which consecutive measurement of a well established network of plots at country level enables evaluation of the changes in forest area, stem volume and net annual stem wood increment. This approach is currently the default method for forest carbon sink evaluation for GHGs reporting within the UNFCCC (Houghton *et al.*, 1997; Lindner & Karjalainen, 2007), and readily enables estimation of the C stock at country level. Nevertheless, some assumptions are sometimes made, such as considering valid biomass expansion factors (BEFs) for all stand age and locations. This implies some uncertainty in the estimation, but on the other hand, the estimation process is easily evaluated because volume information is widely available in most countries from National Forest Inventory programs.

Although this method is widely used and accepted for above and belowground biomass C estimation, C stock evaluation in other compartments, i.e. soil, becomes difficult. Soil C inventories

with re-measured plots are not common; the case of Sweden (Ståhl *et al.*, 2004) can be mentioned as an example. Since soil C measurement is time consuming and implies large uncertainty, huge efforts have been made to develop models for accurate estimation of soil C stocks. Some attempts have been made as regard empirical modelling, considering soil properties, climate conditions or biomass variables as independent variables. Other alternatives include process-based models in which stock or changes in SOC can be predicted from environmental variables, which are not always readily available. The most widely used methodologies for examining changes in the soil are those based on information from National Forest Inventories, which enable estimation of the organic matter provided to the soil from turnover rates of biomass compartments. Adequate estimation of the decomposition rate of these wastes from climatic data enables estimation of the release of CO_2 from microorganism respiration, and also the exchange rate to the mineral soil compartment. Nevertheless, there is a great need for more consistent assessment of empirical data from field studies (Lindner & Karjalainen, 2007), which would enable validation of the models in use for SOC estimation.

Moreover, methods based on measurement of changes in C stock also include smaller scale techniques in which changes in C must be estimated. For example, the changes in the stability of SOM after a perturbation can be assessed by changes in stock. The difference in the proportion of organic compounds before and after disturbance enables estimation of the final effect of the perturbation. If high temporal resolution is required, the remeasurement or chronosequence approach must be applied.

Quantification of C in forests implies a high degree of uncertainty (Grace, 2004), and estimations from different methods may yield different results (Löwe *et al.*, 2000; Houghton, 2003; House *et al.*, 2003). Sometimes these differences are due to different accounting of pools (Houghton, 2003), or difficulties in considering human disturbances like harvesting or other management practices. A common methodology must therefore be followed worldwide to examine changes in global C stocks.

1.3.3. Methods based on production

These methods are mainly restricted to inventory of the C in harvest and wood products (Dias *et al.*, in press). Although some models use a flux-data approach based on lifetime analysis, by assuming lifetimes and associated decay rates for harvested wood products (Tier 2 estimation level, (IPPC, 2003)), estimations at country scale (Tier 3 estimation level, (IPPC, 2006)) are based on the production method, and involve more complex country specific data and methods (Dias *et al.*, in press).

The contribution of harvesting wood products (HWP) in the production approach is the net change in carbon stock of HWP produced from domestically grown wood (Dias *et al.*, in press). For this, country-scale data on wood production, wood consumption for each industry type, industrial

transformation performance, process losses and destination, and average lifespan of wood products are required.

1.4. Research questions

The objective of this doctoral thesis is to discuss the modelling of C accumulation in forest systems, particularly forest plantations, as well as to evaluate the effect of different management practices on C dynamics. The main research questions to be addressed are thus related to these issues, with the aim of improving the tools and models for quantifying carbon stocks, and changes in carbon due to land use changes. This study focused on the three scales at which carbon can be estimated: tree level, stand level and landscape level. As a specific case, the study focused on the estimation of a dynamic process, i.e. the afforestation of former pasture land in northern Spain.

1.4.1. How do tree species affect C dynamics in afforestation of former pasture land? Chapter II

Net primary production differs among biomes, land uses and management schemes (Saugier *et al.*, 2001), as do the equilibrium soil C densities in stand biomass and soil in the steady state. Perturbations such as catastrophic events or changes in land use lead to new environmental conditions, which lead to changes in the equilibrium C density in the system. The direction and intensity of C changes in different pools are determined by C densities in the steady state after the perturbation. However, there is a transitory period before the new equilibrium is reached when the changes in C density are not clear, and where wrong management may lead to a large loss of carbon. This period has not been well studied for some land use changes, and conclusions may provide valuable information about the age at which the initial carbon densities are reached, or the new C density equilibrium level.

While changes in C density in tree biomass are easy to estimate, the changes in C density in soils are not so. This is partly because carbon sequestration in soils are affected by several factors (Lindner & Karjalainen, 2007): (i) litter production, which determines the carbon input into the soil, (ii) decomposition, which releases carbon back to the atmosphere, related to the specific climatic conditions, (iii) chemical litter composition, (iv) soil biological activity, (v) hydrology, (vi) specific conditions for stabilization of organic matter in the mineral soil, and (vii) forest management and disturbances (Jandl *et al.*, 2007). Since most carbon dynamics studies are based on modelling approaches, studies based on empirical data are required for more consistent assessments (Lindner & Karjalainen, 2007). Moreover, the relatively slow changes in soil carbon stock make it difficult to evaluate the changes by use of direct measurements (Conen *et al.*, 2004). Eco-physiological modelling is therefore the most commonly used approach for describing and predicting changes in SOC. However, direct measurement of changes in SOC provides valuable

information for either validating eco-physiological model predictions or developing an empirical model, although it is time consuming and the results do not always enable significant conclusions to be reached because of the high variability.

Statistical analysis of measurement errors indicates that the sample size should be quite large to enable detection of differences in soil C (Ney *et al.*, 2002; Conen *et al.*, 2004; Oliver *et al.*, 2004; Smith *et al.*, 2004). Moreover, it is often difficult to differentiate between management influences, natural stand dynamics, and long term changes in soil carbon accumulation (Lindner & Karjalainen, 2007). This is because some methodologies do not allow correction of local tendencies in SOC, or are biased because of the effect of external conditions (i.e. climate change). Study of the effect of afforested species on the C budget has been seldom addressed and may be of major importance in these effects.

Because of the strong trend observed in north and northwestern Spain, whereby forest plantations are established on former pasture land, the main research questions addressed in Chapter II are:

- ✓ Is the chronosequence technique combined with paired plots an adequate approach for describing the changes in C in biomass, litter and soil in a non steady-state forest?
- ✓ What is the effect of the tree species and the companion vegetation on the system C dynamics during this transitory period?
- ✓ Are fast growing plantations established on former agricultural land managed correctly as regards C sequestration criteria?

1.4.2. How do sampling intensity and explanatory variables affect the predictive ability of tree-level biomass equations? Chapter III

Biomass equations at tree level are constructed from standard trees, which represent the population of interest. Biomass is estimated in this sample of trees, and related empirically to easy-to-measure tree variables (diameter at breast and stump height, and total height are the most common). These variables are more closely related to stem than to crown biomass, but because of increasing interest in obtaining accurate predictions of the crown biomass fraction for research on energetic use and nutrient cycling, the relationships between crown variables (living crown height, maximum crown diameter and living crown basis height) and crown biomass fractions are being studied to develop accurate models for predicting these fractions. Some authors consider crown variables as good predictors of crown biomass fractions (Clark, 1982; Satoo & Madgwick , 1982; Carvalho & Parresol, 2003; António *et al.*, 2007), but this type of study is scarce.

Since direct measurement of biomass involves drying and weighing the whole tree, alternative methodologies have been developed to obtain handy estimations of the dry mass. Ratio type estimators are the most commonly used method for developing biomass equations (Briggs *et al.*, 1987). This approach enables estimation of the dry mass of the individual tree from stem samples or samples of other crown fractions. Ratio type estimators involve collection of disk samples along

the stem to determine the dry mass from the average moisture content and the total fresh weight, or from the stem volume and the average basic density. Studies in which biomass equations are developed at tree level do not usually provide information about the exact position of the samples taken and their distribution. Moreover, in such studies samples are usually taken systematically from stump height, because of the accessibility of this position and that fact that no commercial logs are wasted. However, the effect of this on the estimation of the dry mass is still not clear.

The questions addressed in Chapter IV are:

- ✓ What is the effect of sampling intensity on the dry mass estimation of sampling trees when systematic sampling and ratio type estimators are applied?
- ✓ Do crown variables improve the accuracy of crown fraction biomass equations?
- ✓ What is the ability of biomass equations to predict the proportion of biomass fractions?
- ✓ Is there a systematic bias inherent in the bole sampling procedure?

1.4.3. Are *Eucalyptus* plantations suitable for carbon sequestration and bioenergetic purposes at the stand densities conventionally used in southern Europe? Chapter IV

The effects of forests as regards climate change mitigation may be derived from product yield, bioenergy substitution and C stocks in biomass and soils (Canadell & Raupach, 2008). Product yield is usually expressed in terms of harvested or produced volume, whereas bioenergy substitution is usually expressed in terms of energy, or tonnes oil equivalent (TOE). This depends on the transformation procedure of the biomass in question. When energy is transformed by combustion it is the high heating value (HHV) of each biomass compartment that determines the transformation performance. When the biomass is transformed to second generation biofuels (i.e. bioethanol), it is the cellulose content that determines the transformation rate to this type of energy. The TOE in each case depends on the source of energy substituted, while the same amount of energy can be obtained for combustion of several types of combustible; as a convention, this must be expressed in terms of the energy released by the combustion of one Mg of oil ($4 \cdot 10^{10}$ J), which is equivalent to a 1.4 Mg of coal, 4.5 Mg of lignite and $10 \cdot 10^3$ m^3 of natural gas.

The traditional way of estimating this type of bioenergy production is from volume or biomass (Fig. 1.2). This implies a second estimation phase in which the energetic yield value is estimated from volume or biomass. A direct relation of each one of these forms of energy or carbon by specific models may avoid concatenating errors. Moreover, this would allow more accurate comparison of several management alternatives or the use of particular species for energy purposes.

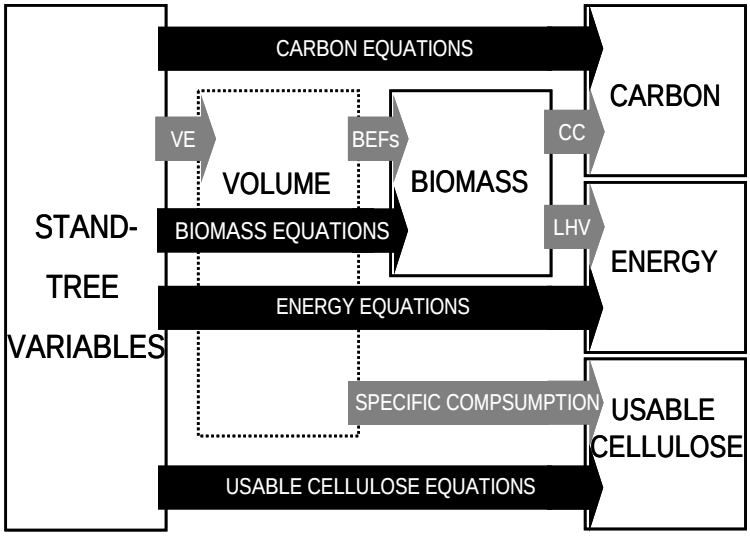

Figure 1.2. Process of biomass, carbon, energy and usable cellulose estimation from stand or tree variables. VE: volume equations; BEFs: biomass expansion factors; CC carbon concentration in biomass; LHV low heating value.

Most studies that compare species or management alternatives for short rotation woody crops (SRWC) are based on a small number of plots (Mitchell *et al.*, 1999; Sims *et al.*, 1999b; Dickmann, 2006), sometimes a few trees, and usually established at very high densities. As thinning is not applied in SRWC management, static growth models may be suitable for yield and bioenergy production modelization, although such studies are still scarce and focus on species used for SRWC in northern Europe. Because of the large installation costs of these high density crops, the requirements of some dimensional constraints for harvesting and production of second generation combustibles, and the low implementation of this type of crop in the north of Spain, the profitability of the current management schemes for bioenergy production is being investigated. This is important because it represents the actual potential to produce biomass without substantial changes to existing management schemes.

The questions addressed in Chapter IV are:

- ✓ How well are standard low density plantations of *Eucalyptus* adapted for bioenergy purposes and carbon sequestration in northern Spain?
- ✓ How do harvesting limitations as regards tree size affect stand management in short rotation woody crops of *Eucalyptus*?
- ✓ How wide is the management window as regards self thinning for each initial stocking density?

1.4.4. How does soil organic matter nature change during afforestation of former pasture land? Chapter V

Although the first step in describing SOC dynamics after land use change is to examine changes in total SOC, there are large differences among SOM compounds regarding stability,

which must therefore be studied in greater detail. Although there are some studies about the effect of climate change on the chemical stability of soil organic matter compounds (Bellamy *et al.*, 2005; Kirschbaum, 2006), there is an important gap in the knowledge about the chemical nature of the SOC following land use change. While information about total SOC may suggest that all C released after land use change occur in the most labile fractions, it is known that degradation of recalcitrant organic matter readily takes place in upper horizons in which some stabilization processes are less active than in deeper horizons (von Lützow *et al.*, 2006). Moreover, more detailed work is necessary to examine changes in this type of process, since the traditional classification of SOC into labile and recalcitrant is not sufficient for describing the SOC stability and dynamics.

Traditional techniques for examining changes in chemical SOC stability include ^{13}C CP-MAS NMR, Fourier transform infrared spectroscopy and pyrolysis/GC-MS. Although these methods are highly reproducible and accurate, they are also expensive and time consuming. However, some studies on SOM dynamics, especially those focused on modelling requires analysis of a large number of samples, while at the same time offering a high degree of reproducibility and accuracy. In this sense, calorimetry and thermal analysis are rapid, inexpensive techniques that provide information about the stability of SOC and have already been tested in steady state systems. Nevertheless, accurate methods of describing dynamic processes are being investigated.

The questions addressed in Chapter V are:

- ✓ What fractions of SOC are affected by the losses when large losses of SOC occur in the soil?
- ✓ How suitable are calorimetry and thermal analysis for detecting SOC stability changes in forest soils?

1.4.5. How do management practices affect the mitigation effect of fast growing plantations established on former pasture land? Chapter VI

Prediction through empirical models is restricted to unchanged conditions rather than to adjusted conditions (IPPC, 2006). This also implies that management practices are considered to be the same as those observed in the population modelled. The equilibrium between management intensity, C stock and mitigation effect is difficult to assess. Although excessive harvesting schedules can lead to severe damage to forest ecosystems, under-harvesting can lead to under use of the stand potential, and maintenance of the forest at more unstable stages of development, as regards e.g. fires, storm events and diseases. This must be considered for maximizing the potential of forests as regards climate change mitigation. The effect of several management practices on changes in forest C accumulation must be assessed with models that are sensitive to this type of change.

In this sense, the CO_2Fix model (Nabuurs *et al.*, 2002; Masera *et al.*, 2003; Schelhaas *et al.*, 2004) enables estimation of the changes in C stocks and fluxes by use of the full carbon

accounting approach. This model is divided into six modules: biomass, soil, products, bioenergy, financial and carbon accounting. Changes in C in all modules are driven by current annual increments in the species considered, and also by the climatic data and the initial conditions regarding C stock. The usefulness of the model has already been demonstrated for even and uneven-aged forest stands worldwide (Schelhaas *et al.*, 2004; Groen *et al.*, 2006), enabling comparison between different management practices and also initial conditions.

Since there are no either purely eco-physiological or empirical models (Landsberg & Sands, 2011), estimations for some modules of the model used are obtained from empirical data. Although previous studies of the sensitivity of the chosen model in regard to the input data indicates that yield tables are one of the most important parameters (Nabuurs *et al.*, 2008), different models are sometimes available and direct measurements are not always available for validation. Three different models were therefore used to produce yield tables and were validated with measured data. This same process was carried out with the soil module, since there was not sufficient information about its usefulness for southern European conditions. Moreover, there is not enough information about the mitigation effect of forest products available on the northern Spain market.

The questions addressed in Chapter VI are:

- ✓ How important are accurate yield tables for describing C accumulation on biomass by using the CO_2Fix model?
- ✓ How well does the soil module of the CO_2Fix model (Yasso model) enable estimation of the changes in C in forest soils in northern Spain, and how sensitive is the module to turnover rate parameters?
- ✓ How do site conditions and management alternatives affect the C accumulation effect in fast growing plantations established on former pasture land?
- ✓ What is the contribution of forest products and bioenergy substitution to the mitigating effect of the forest plantations in northern Spain?

1.5. Objectives

The overall objective of this thesis was to develop methods and tools for estimating carbon stocks in biomass and soil in *Pinus radiata* (D.Don), *Eucalyptus globulus* (Labill) and *Eucalyptus nitens* (Dean & Maiden) Maiden plantations over former agricultural land in north-western Spain.

The specific objectives were:

- ✓ To evaluate carbon accumulation in biomass, litter and soil on former agricultural land afforested with different species (Chapter II).

- ✓ To develop biomass equations at tree level for *E. nitens* plantations, and to evaluate the effects on tree dry mass estimation of the sampling intensity, sampling methodology and independent variables considered (Chapter III).
- ✓ To evaluate the energetic and carbon sequestration ability of *E. globulus* and *E. nitens* plantations in short rotation woody crops (Chapter IV).
- ✓ To evaluate the stability of the soil organic matter after land use change from pasture to forest plantation with fast growing tree species (Chapter V).
- ✓ To evaluate the mitigation effect of several management regimes for the tree species studied, considering the whole product cycle and the stock effect on soil and biomass (Chapter VI).

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Chapter II

Influence of tree species on C sequestration in afforested pastures in a humid temperate region

2. Influence of tree species on C sequestration in afforested pastures in a humid temperate region

Abstract

This study examines the influence of tree species in relation to biomass and soil C dynamics in plantations established on former pasture land. Data on the C sink capacity of such plantations will provide valuable information for designing improved management strategies for afforestation programmes aimed at mitigating CO_2 emissions.

The study was carried in the temperate forest of southern Europe, one of the most productive timber production systems in Europe. The study, designed to control most of the variability at regional level, involved a network of 120 paired plots (former pasture land-new plantations of different ages) established to construct three well-replicated chronosequences of the most common tree species in humid temperate systems.

The mean rates of C sequestration (biomass and soil) estimated throughout the rotation ranged between 8.7 and 14.6 Mg C ha^{-1} yr^{-1} (*Eucalyptus nitens* > *Eucalyptus globulus* > *Pinus radiata*), and the contribution of the soil (litter plus mineral soil) ranged from 8 to 18% (*Eucalyptus nitens* > *Pinus radiata* > *Eucalyptus globulus*). The humid temperate climate and the sandy loam texture of the soils favoured large losses of SOC from the uppermost mineral soils during the 10 yr after afforestation. The higher loss of SOC in the pine soil (26% of initial SOC) than in the eucalypt soil (19.5% of initial SOC) was attributed to the lower transfer of organic C to the mineral soil, as a result of the lower litter decomposition rate and the lower belowground litter input from associated vegetation. The climate also favoured the rapid development of tree biomass and subsequent C sequestration in biomass and soils.

The C sink capacity of forest plantations can be maximized by elongating the rotation length and adopting suitable management strategies for each species. This is especially important in intensive forest plantations in which the high intensity of harvesting may prevent accumulation of SOC in the long term.

Keywords: Eucalypt, pine, pasture, afforestation, tree biomass, litter, soil organic matter.

2.1. Introduction

The change in land use that is taking place in many areas of the world has a major impact on the world's terrestrial carbon (C) balance and is therefore a key factor in the mitigation of climate

change (IPPC, 2007). In the tropics, extensive areas of primary forest are being converted to agriculture land to meet the current food demand. However, in Europe, North America and Russia, traditional agriculture practices have been abandoned and large areas of cropland and pasture land are being transformed to forests. In Europe, this phenomenon has been favoured by the implementation of EEC directive 2080/92 (EU 1992), which laid down a grant scheme for promoting reconversion of agricultural land to forest, with the aim of reducing surplus agriculture production (Stanturf & Madsen, 2002), although at the same time it may have contributed to balancing the C by enhancing the C sink capacity of terrestrial ecosystems.

The rate of accumulation or loss of soil organic carbon (SOC) following land use change is governed by the balance between C inputs and C decomposition rates. This balance becomes negative as the intensity of land management increases from forest to grassland to cropland. However, the magnitude and progress of the changes in C contents following changes in land use are highly variable because of the influence of different factors, such as net primary production and rooting patterns (Jobbágy & Jackson, 2000), quality of organic inputs (Berg, 2000), certain soil properties (nutrient availability, texture, (Golchin *et al.*, 1994; Mendham *et al.*, 2003)) and the intensity of past and present management (Balesdent *et al.*, 2000).

Although the conversion of natural forest land to cropland produces clear and rapid losses of biomass C and SOC (Mann, 1986; Schlesinger, 1986; Oades, 1988; Vitorello *et al.*, 1989; Johnson, 1992; Davidson & Ackerman, 1993; Smith *et al.*, 1993; Schimel, 1995; Bashkin & Binkley, 1998; Paustian *et al.*, 2000; Post & Kwon, 2000; Walker & Desanker, 2004), the effect of the opposite change in land use on SOC is slower, and C recovery is not universally observed, at least in the short term. Nevertheless, data on the changes in SOC following conversion of forests to pasture are widely variable, and while some authors observed reductions in SOC of 20-30% (Detwiler, 1986; Veldkamp, 1994; Guggenberger & Zech, 1999; Glaser *et al.*, 2000; Rhoades *et al.*, 2000), others did not find significant changes (Reiners *et al.*, 1994; Bell *et al.*, 1995; Corre *et al.*, 1999; Mendham *et al.*, 2003) and yet others reported increases (Lugo & Brown, 1993; Neill *et al.*, 1997; Fearnside & Barbosa, 1998). Murty *et al.* (2002) and Guo & Gifford (2002) carried out a meta-analysis of this land use change worldwide, and reported that conversion of forest to uncultivated grazed pasture land does not generally lead to loss of SOC, although individual sites may lose or gain soil C, depending on specific circumstances, such as the application of fertiliser or removal of plant residues. This demonstrates the great potential of pastures to sequester SOC, mainly because of the large inputs of C via herbaceous root litter (Guo *et al.*, 2007), which may lead to more C accumulation in upper mineral soil than in forests (Jackson *et al.*, 2002).

There is great uncertainty about the changes in SOC associated with afforestation of pasture land, because of the high SOC content of the latter. Afforestation of this type of land therefore usually leads to decreases in SOC in the short or medium term (Laganière *et al.*, 2010; Poeplau *et al.*, 2011), and sometimes no variations are observed in the long term (Marín-Spiotta *et al.*, 2009; Poeplau *et al.*, 2011). This becomes more important when the species used in afforestation are fast growing species with a short rotation length, in which recovery of SOC may not occur by the

end of the rotation. The afforestation may therefore result in high temporal C losses via soil respiration in comparison with the C inputs by litter. Some authors have suggested that this is a temporary effect, in which only the labile C pool is exhausted (Cerli *et al.*, 2008; Huang *et al.*, 2011). However, recent evidence shows that degradation of recalcitrant SOM can also occur in the uppermost soil layers (von Lützow *et al.*, 2006, Chapter V). It is not clear whether these initial losses are compensated in fast growing, intensively managed species.

The C balance after afforestation is greatly affected by the tree species, as a result of differences in growth rates of the trees. In addition, litter production and litter quality, which are greatly influenced by tree species, have a strong influence on the SOC dynamics (Berg, 2000; Vesterdal *et al.*, 2008). However, since most of the current knowledge is based on studies of afforestation with coniferous species (Berthrong *et al.*, 2009), the influence of the tree species on SOC dynamics has not yet been accurately evaluated.

In studies that have attempted to evaluate the effect of tree species on afforested land, the differences in SOC dynamics are attributed to the influence of the different turnover rates of the litter (Vesterdal *et al.*, 2008), the cover and type of ground vegetation (Lemma *et al.*, 2006), or both (Paul *et al.*, 2002; Huang *et al.*, 2011; Kasel *et al.*, 2011). The influence of N-fixing tree species has been also be recognized (Nilsson & Schopfhauser, 1995; Kasel *et al.*, 2011), as a higher yield in poor soils leads to higher organic matter inputs to the soil, and also improves litter quality and the speed of decomposition of OM (Conteh *et al.*, 1997). Recent studies have shown that these different sources of litter inputs can even lead to changes in the SOM composition (Huang *et al.*, 2011; Chapter V).

The capacity of the soil to act as a C sink is also influenced by how rapidly the litter converts C into humus (Silver *et al.*, 2004; e.g. Kanerva & Smolander, 2007; Prescott, 2010). The decomposability of the litter not only depends on its chemical composition, but also on microclimatic conditions determined by the different canopy development and stand structure. Moreover, the same microclimatic conditions have a direct influence on the cover and type of ground vegetation, which in turn alters the amounts, composition and type of litter (Ostertag *et al.*, 2008; Berg *et al.*, 2009). This is particularly important because roots incorporate more C into the soil than litter layer material (Jones *et al.*, 2009). Thus, ground vegetation dominated by grass species incorporates C rapidly into soil organic matter because the root system develops quickly (Andrade *et al.*, 2008; Laungani & Knops, 2009).

The silvicultural parameters most closely related to SOC dynamics are: tree species, site preparation techniques, initial stocking, rotation length and other parameters more specific to each type of tree species management (pruning, clearcuting, thinning, application of fertilizer, etc.) and autoecology. These aspects have been discussed by Jandl *et al.* (2007) and, in the case of the species considered here, by Balboa-Murias *et al.* (2006). In addition to management practices, a comprehensive representation of the entire forestry sector system should be considered, taking into account the C pools and fossil fuel substitution, although the latter pool has not been considered in the international agreements on reduction of emissions (UNFCCC, 2002). All of these

factors are particularly important in fast growing tree species, in which intensive management in short rotations, as well as harvesting of logging residues may prevent accumulation of SOC in the long term.

Most studies concerning the effect of different species and types of management on C sequestration in afforested land have compared the C stocks in several pools in pasture land and forest plantations in the steady state, rather than considering the dynamic changes that take place. In many cases, the shifts in key parameters throughout the rotation, such as tree growth, stand structure, associated vegetation and litter development are not considered. Thus, the temporal dynamics not only provide an understanding of the different mechanisms of C sequestration after afforestation, but are also useful for designing the most appropriate type of management to maximize the C sink capacity.

Most approaches evaluating the capacity of soils as C sinks focus on plot level, and few studies have been extended to landscape levels (Johnston *et al.*, 1996; Turner & Lambert, 2000; Conant *et al.*, 2003). Such studies show the high degree of variability in the SOC dynamics following afforestation, even under rather homogeneous conditions. This high variability emphasizes the risk of making erroneous conclusions about SOC dynamics when the experimental design does not take this variability into account (Berg *et al.*, 2009). A correct methodology must ensure adequate sampling replication at plot level, and proper sample analysis to take into account most of the variability for extrapolation of the results of plot level studies to a larger scale (Goidts *et al.*, 2009). Methodological procedures for quantifying the changes in SOC after afforestation are: (i) paired sites, (ii) chronosequence studies, (iii) multiple re-sampling, and (iv) process and modelling studies (Turner & Lambert, 2000).

Chronosequence studies use a series of plots in plantations of different ages with supposedly similar management regimes and environmental conditions, and translate spatial differences between soils into temporal differences (Huggett, 1998). Although the chronosequence approach cannot replace long-term field experiments, there are certain disadvantages with the latter, such as the delay in obtaining results, workload and particular trends in external parameters (i.e. climate and local conditions), which may cause a systematic bias in the observations. The paired-plots approach is an alternative method in which one of the paired plots represents the initial conditions. Because of the high spatial variability in SOC measurements (Johnston *et al.*, 1996; Turner & Lambert, 2000; Conant *et al.*, 2003), the combined use of chronosequences and the paired-plots approach may provide a suitable way of detecting changes in soil pools, and of correcting local tendencies.

The objectives of the present study were: (i) to examine the influence of tree species on the C dynamics in the forest system (tree biomass, litter and mineral soil) following afforestation of pasture land, (ii) to explore the relationships between tree biomass development on litter and SOC dynamics in three forest plantations established on former pasture land, and (iii) to evaluate the C sink capacity of the different types of plantations in relation to management. A specific methodology for sampling and data management was designed to restrict most of the variability on

a regional scale and therefore to provide accurate information on C dynamics in the different tree species planted. The design was applied to temperate forest plantations of southern Europe, one of the most productive timber production systems in Europe, where important afforestation processes may play an important role in mitigating CO_2 and other GHGs emissions. The data obtained in this study will provide valuable information about the effects of such afforestation programmes on C sink capacity.

2.2. Materials and methods

2.2.1. Site description and experimental design

The study was carried out in Galicia (NW Spain), in an area of 7000 km^{-2} representative of the Atlantic-climate zone of northern Spain. The 20 year annual average rainfall in the area is 1378 mm (range 884-2107 mm), and the temperature, 12.1ºC (range 10.3-14.8ºC). The wettest month is November, with an average rainfall of 160 mm, and the driest August, with 44 mm. The lowest mean monthly temperature 7.1ºC occurs in January, and the highest 18.2ºC, in August. The soil humidity and temperature regimes are Udic (mean period with partial drought, 1 month) and Mesic (mean frost-free period, 10 months), respectively. The soils were developed from granitic rocks, schist and shale, and classified as Humic or Distric Cambisols and Alumi-humic Umbrisols (IUSS Working Group WRB, 2006). The soil has a loam or sandy loam texture and is well drained.

The average values for selected characteristics of the afforested plantations studied are shown in Table 2.1. In all plots, the site qualities were higher than in plantations established on former forest soils, probably because of the better quality of the soils (soil depth, stoniness, higher water supply) and prior fertilization. A network of 120 paired plots, made up of former pasture plots and afforested plantations, was established. The plots were distributed in three sets of 40 plots planted with the most commonly used species in the area: *Eucalyptus globulus* Labill, *Eucalyptus nitens* (Deane & Maiden) Maiden and *Pinus radiata* D. Don. Each set was an independent chronosequence in which the range of ages covers the usual rotation lengths applied to these plantations, thus enabling conclusions to be reached as regards the effects of the single-stem rotation following land use change: 1-23 years for *E. globulus*, 2-18 years for *E. nitens* and 2-40 years for *P. radiata* chronosequence respectively.

In all cases the prior use was as pasture land, in which low intensive management was applied for at least 25 years (according to landowners), and some of which has recently been afforested. The plots were dominated by a mixture of *Lolium multiflorum*, *Lolium perenne*, *Trifolium pratense*, *Trifoliun repens* and *Dactylis glomerata*, although as the time since last perturbation increased, *D. glomerata*, *Agrostis capillaris* and *Holcus lanatus* became more predominant. The pastures are normally harvested for silage 1-2 times a year, and grazed once or twice a year. They are usually

renewed every 8-10 years by rotovating to a depth of 20 cm. In all cases, afforestation was carried out after ripping at 50 cm depth and planting along the row, so that soil disturbance was considered low. No fertilization or weed control was carried out in the plantations. The pastures were selected so that time since last tillage was more than four years. The minimum size of each grassland and afforested plantation was 0.5 ha.

To ensure that all sites were similar as regards soil type and land use, selection of the study sites was based on direct observation of the terrain in adjacent pastures, and consultations with landowners. The plantation age was established using an increment borer to sum the ring number for *P. radiata* and *E. nitens*, which was clearly visible and easy to assign to yearly growth periods, and considering the planting date declared by the owner to the Forestry Administration for *E. globulus*.

Table 2.1. Main site characteristics (average and standard deviation) of the stands studied.

	Units	*E. globulus*	*E. nitens*	*P. radiata*
Number of stands	(n)	40	40	40
Age interval	(yr)	1-23	2-18	2-40
Stand density	(stems ha^{-1})	1108 (309)	1011 (258)	1146 (410)
Bed rock (Granitic rock /Schist-Slates)	(n)	8/32	5/35	25/15
Site Index*	(m)	23.3 (6.5)	15.3 (4.4)	24.8 (4.4)
Altitude	(m)	242 (173)	517 (63)	466 (115)
Average annual Temperature	(ºC)	13.3 (1.0)	11.6 (0.5)	11.5 (0.8)
Accumulated annual precipitation	(mm)	1488 (377)	1434 (322)	1213 (219)

*Reference ages for site index were 10, 6 and 20 years for *E. globulus*, *E. nitens* and *P. radiata* respectively.

Similar forest management regimes, in terms of site preparation, source of seedlings, and pruning and harvesting regimes, were applied in all afforested stands. Stands of *E. globulus* were located in coastal areas at altitudes below 300 m.a.s.l., whereas *E. nitens* and *P. radiata* stands were located in the inner area, generally between 300 and 500 m.a.s.l. As the selected stands were similar in regard to climate, soils, tree species, prior land use and stand management, the only theoretically difference assumed among plots was the age since afforestation.

2.2.2. C determination in above ground tree biomass

For determining carbon density (Mg C ha^{-1}) in aboveground biomass, diameters at breast height (to the nearest cm) and total height (to the nearest dm) were measured in all trees in circular plots of radius 10 m. Dry weight of aboveground biomass was estimated using the equations proposed by Merino *et al.* (2005) for *E. globulus*, developed in Chapter III for *E. nitens*, and by Balboa-Murias *et al.* (2006) for *P. radiata* chronosequences. The carbon concentrations in

each biomass fraction were those reported by the latter authors, except for *E. nitens*, for which the values reported in Chapter IV were used.

2.2.3. Soil sampling and analysis

For sampling the soil (litter and mineral soil to 30 cm depth), a 50 x 50 m plot was selected within each stand, at a distance of more than 30 m from the edge of the stand. Five samples per plot were taken from between tree rows to minimize any disturbance from the site preparation.

For sampling the litter layer, 0.3 x 0.3 m squared frames were thrown at random within each plot, on 5 occasions. All aboveground soil litter was collected and dried at 40ºC until constant weight. Carbon contents in the litter were measured for *E. nitens* (47.9%C), whereas already published data were used for *E. globulus* and *P. radiata* (Merino *et al.*, 2005).

For mineral soil, three soil layers were considered at depths of 0-5, 5-15 and 15-30 cm. The first two correspond to the A horizon, and the deepest layer A2, AB or BA. Sub-samples of the mineral soil layer were collected with a steel corer, and were combined to form one bulk sample per plot. These samples were oven-dried at 40ºC, sieved at 2 mm and the stoniness was determined. In the fine soil fraction, total C and N were analyzed with a LECO Elemental analyzer, whereas soil particle analysis in the upper 15 cm was performed by laser diffractometry, with a Mastersizer 2000 diffractometer.

At each sampling point, five density corers were collected in a 100 cm^3 metal cylinder, which was oven-dried at 105ºC and weighed to determine the bulk density following the methodology of Blake and Hartge (1986). The C content in each layer was determined by expression [2.1], where *CD* is the carbon density in each layer (Mg ha^{-1}), *CC* is the carbon concentration in each layer (as a fraction of unity), *Db* is the bulk density (g cm^{-3}), *T* is the thickness of each layer (cm) and *S* is the stoniness (as a fraction of unity).

$$CD = CC \cdot Db \cdot T \cdot (1 - S) \cdot 100 \qquad [2.1]$$

2.2.4. Evaluation of C sequestration in the forest system

The amounts of C in aboveground biomass and organic layers was considered as net gain relative to pastures, and therefore only absolute values are reported or plotted against time since afforestation. For modelling of aboveground biomass C changes with time since afforestation, the Richards (1959) model was used to describe the relationship between aboveground biomass carbon (C_W, Mg ha^{-1}) and stand age (*t*, yr), shown in equation [2.2]. The model for each species was fitted with the MODEL procedure of the SAS/ETS® system (SAS Institute Inc, 2004). The root of mean square errors (*RMSE*) and adjusted determination coefficient (*Adjust*. R^2) were calculated for each model fit.

$$C_W = b_0 \cdot \left(1 - e^{-b_1 \cdot t}\right)^{b_2} \quad [2.2]$$

Carbon sequestration in each mineral soil layer was evaluated in absolute terms as the difference between the forest subplot CD_F and the pasture subplot CD_P (carbon absolute difference *CAD,* Mg ha^{-1}), and in relative terms as the difference in percentage of carbon density (carbon relative difference, *CRD*, % of initial carbon density), with expression [2.3]. Both parameters were represented in each plot against time since afforestation to evaluate the changes in soil carbon with time since afforestation.

$$CRD = \frac{CD_F - CD_P}{CD_P} \cdot 100 \quad [2.3]$$

To describe changes in soil C over time, in previous studies on changes in mineral soil carbon after secondary succession, an empirical modelling approach including the gamma function was used successfully (Covington, 1981; Zak *et al.*, 1990). However, in the present study the changes are expected to follow a different pattern, as we hypothesized that the C equilibrium level is different in pasture than in afforested land, and the gamma is function biologically inconsistent in such cases. Alternative models include more parameters than the gamma function, making convergence of the parametric model fit difficult, although this will depend on the amount of data available. In this case, parametric curve fitting procedures did not converge because of the large number of parameters needed to fit a model that adequately captures the apparent shape of the data. Thus, nonparametric fitting was carried out to describe the general trend in the changes in litter and mineral soils. The LOESS procedure in the SAS/STAT (SAS Institute Inc, 2004), was used to divide residuals of the nonparametric curve into ten age intervals, and the 95% confidence levels were determined.

Data were also analyzed by analysis of variance with the GLM procedure of SAS/STAT (SAS Institute Inc, 2004). The Tukey test was used to detect differences between means, considered significant $p<0.05$.

2.3. Results

2.3.1. Changes in the C accumulated in biomass throughout the rotation

Results of non linear fit of aboveground biomass C density are shown in Table 2.2. All parameters were significant at $p<0.005$, and the accuracy of the statistics was adequate. The average changes in the C accumulated in the tree aboveground biomass for the three tree species studied throughout the rotation are shown in Fig. 2.1. The tree growth rates followed the order *E.*

nitens > *E. globulus* ≥ *P. radiata*. The three species continued accumulating C at high rates, even after the common rotation lengths in the region (10-15 years for both eucalypts and 30-35 years for *P. radiata*), which indicates the large potential of the biomass of these species as a C sink.

Table 2.2. Aboveground biomass carbon model parameters and fits for age since afforestation.

Specie	Parameter estimate (Std. error)			*RMSE*	*Adjust.* R^2
	b_0	b_1	b_2		
E. globulus	520.3 (6.23)	0.0589 (0.0021)	2.356 (0.0325)	3.0331	0.718
E. nitens	784.1 (10.41)	0.0393 (0.0013)	1.815 (0.0218)	2.1483	0.835
P. radiata	1569.5 (13.75)	0.0126 (0.0008)	1.466 (0.0201)	4.3544	0.788

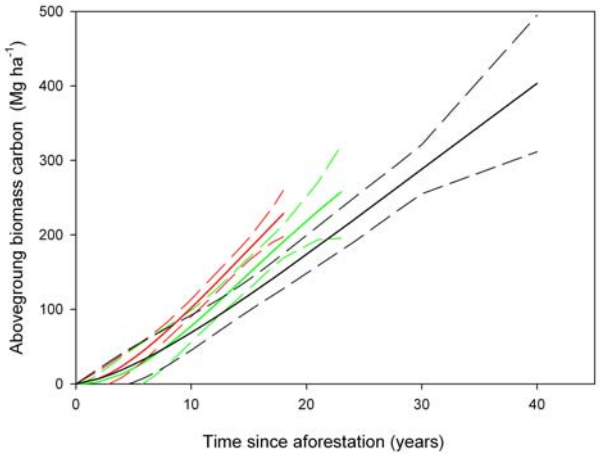

Figure 2.1. Changes in total aboveground biomass C throughout the rotation. Continuous lines indicate the fitted Richards model; dotted lines are 95% confidence levels for the mean. *E. nitens* red; *E. globulus* green; *P. radiata* black.

2.3.2. Changes in C accumulated in litter throughout the rotation

The changes in the C accumulated in the litter layer (and mineral soil to depth 15 cm) for each of the three species are shown in Fig. 2.2. The average trends throughout the rotation were fitted by the nonparametric procedure described in point 2.2. The three species showed different patterns as regards the dynamics and the amounts of C accumulated at the end of the rotation. Thus, in accordance with the higher growth rates of biomass, litter accumulation occurred earlier (2 yr after establishment) in *E. nitens* than in *P. radiata* and *E. globulus* (4-5 yr after forest establishment). The litter C accumulation rates followed the order: *E. nitens* > *P. radiata* > *E. globulus*. Litter accumulation was lower than expected in *E. globulus* stands, considering the high aboveground tree growth. The correlations between the changes in crown biomass and litter layer dynamics were different for each species, and were generally rather weak (n.s. for *E. globulus*; R^2= 0.40 for *E. nitens*, and R^2= 0.55 for *P. radiata*).

Litter accumulation stabilized 10 years after afforestation by both *Eucalyptus* species, whereas the litter layer continued to increase in mature *P. radiata* plantations. At the end of the rotation, the average amounts of C in the litter layer ranged from 10.1 (*E. globulus*, 10 yr), to 24.8 Mg ha^{-1} (*E. nitens*, 10 yr) and 50.9 (*P. radiata*, 35 yr).

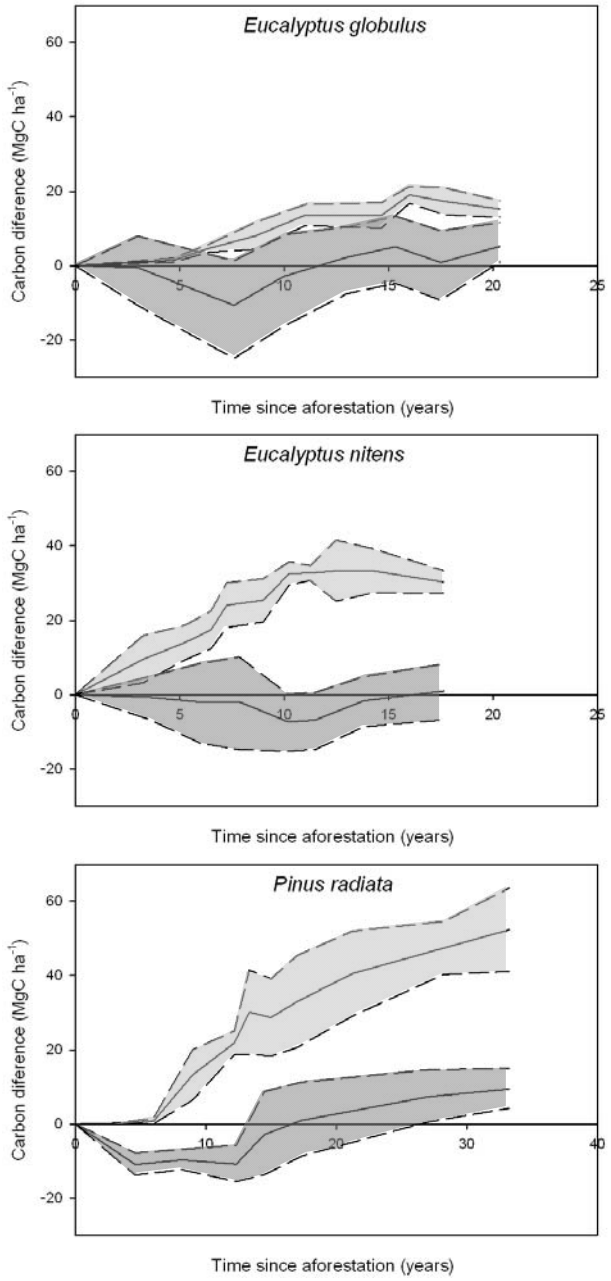

Figure 2.2. Changes in carbon density accumulation (Mg ha^{-1}) in the litter layer and mineral soils throughout the first rotation after afforestation. Dark shaded area: 95% confidence limits for 0-15 cm depth mineral soil (*CAD*); light shaded area: 95% confidence limits organic layer (n= 40 for each of the three species).

2.3.3. Changes in SOC in the mineral soils after afforestation

The changes in SOC density relative to that of the paired pasture (*CRD*) in the mineral soil for each soil depth layer, considering each species separately and together, are shown in Table 2.3. In the first 10 years after afforestation, losses of C in the 0-15 cm layer were found in all three tree species, ranging between -52.0% in *P. radiata* to about -0.2% in both species of *Eucalyptus* in the first 5 years. However, the ANOVA only revealed significant changes in the 0-5 and 5-15 cm soil layers under *P. radiata*, which emphasizes the high variability in the *Eucalyptus* stands. In the 11-15 yr period, the SOC contents were similar in afforested and pasture soils for all species. Finally, in the stands older than 20 yr (only for *P. radiata*), the SOC contents were significantly higher in the uppermost soil layer (0-5 cm) than in the other layers. No such trend was detected in the 15-30 cm layer.

Table 2.3. Average values (and standard deviations) of relative difference in carbon density (*CRD, %*) considering all species jointly (n= 120), and each species separately (n=40). Significant differences for a given soil depth are indicated by different letters.

	T (years)	Mineral soil layers (cm)									
		0-5		5-15		15-30		0-15		0-30	
All species	0-5	-21.1 (26.7)	a	-6.9 (52.8)		1.3 (42.3)		-14.6 (36.8)		-9.5 (33.9)	
	6-10	-6.2 (31.2)	ab	3.5 (40.6)		10.2 (45.8)		-2.0 (31.7)		1.5 (33.1)	
	11-15	-3.3 (38.4)	ab	8.7 (73.4)		6.4 (64.8)		0.8 (47.9)		2.0 (51.8)	
	16-20	16.6 (49.7)	bc	-4.1 (33.8)		-3.2 (44.4)		1.2 (28.6)		-1.1 (31.0)	
	>21	30.7 (29.7)	c	7.8 (29.1)		3.8 (45.9)		15.5 (23.3)		9.9 (27.0)	
E. globulus	0-5	-4.1 (17.8)		12.3 (43.6)		28.4 (46.4)		-0.1 (22.0)		13.4 (37.6)	
	6-10	-9.4 (34.8)		-9.0 (45.1)		-4.1 (57.9)		-9.2 (39.6)		-8.4 (43.6)	
	11-15	5.4 (45.4)		19.2 (110.4)		4.7 (84.2)		6.8 (66.5)		6.9 (72.7)	
	16-20	8.7 (30.0)		-0.1 (38.7)		-2.5 (42.8)		3.2 (24.3)		-1.4 (30.2)	
E. nitens	0-5	-3.1 (18.5)		13 (34.8)		19.6 (37.4)		-0.3 (24.6)		8.0 (27.9)	
	6-10	-6.0 (29.8)		3.4 (41.7)		-17.3 (46.8)		-1.4 (26.1)		-8.6 (31.9)	
	11-15	-8.9 (22.4)		-6.3 (29.5)		5.0 (62.8)		-8.9 (21.6)		-4.5 (33.7)	
	16-20	29.9 (71.6)		-12.3 (13.8)		-28.2 (20.2)		6.6 (39.9)		-7.4 (26.0)	
P. radiata	0-5	-51.3 (18.6)	a	-52.8 (16.5)	a	-5.3 (29.5)		-52.0 (16.7)	a	-34.1 (17.3)	a
	6-10	-23.6 (29.4)	ab	-23.4 (13.1)	ab	-4.5 (18.8)		-24.1 (10.6)	ab	-17.1 (9.0)	ab
	11-15	-14.3 (41.8)	ab	-16.8 (30.9)	ab	-13.8 (31.0)		-15.6 (32.6)	ab	-16.0 (29.7)	ab
	16-20	32.5 (65.2)	bc	-4.4 (28.8)	bc	4.6 (47.3)		4.6 (31.4)	bc	3.7 (32.6)	ab
	>21	28.4 (29.3)	bc	13.4 (28.7)	bc	10.1 (50.5)		18.9 (23.2)	cd	14.7 (27.8)	b

This effect was also observed in relation to the number of plots that are in a certain group of losses or gains with respect to *CRD*, shown for soil depths of 0-15 and 15-30 cm, and considering all species together (Fig. 2.3). Although the data revealed a high degree of variability, both representations show a general trend for the SOC content in the 0-15 cm soil layer. Carbon was

lost from this soil layer during the first 10 years after afforestation, although gains were observed thereafter. Net positive gains were found from 20 years onwards.

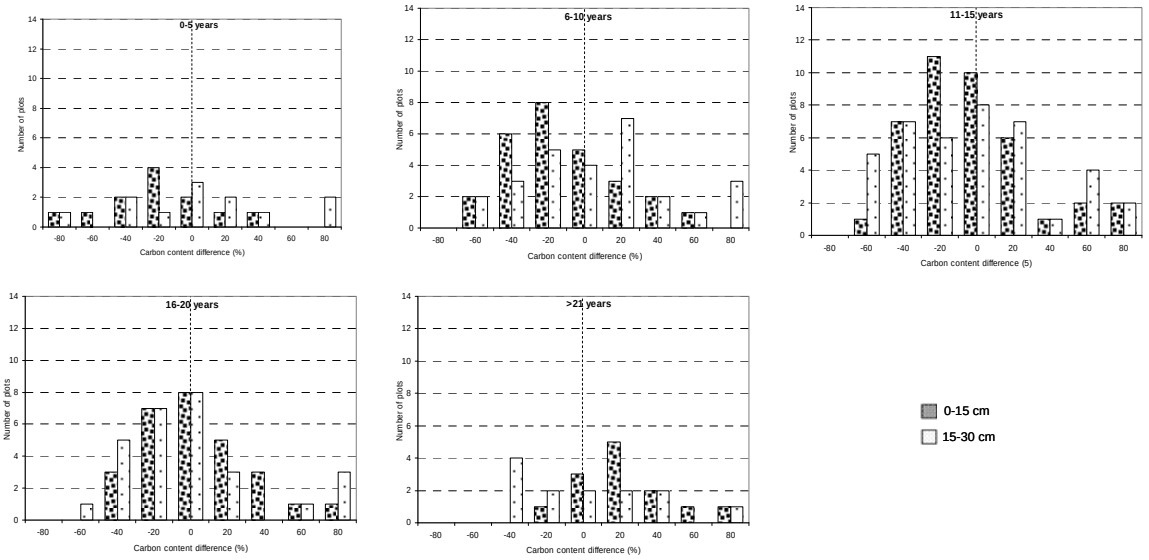

Figure 2.3. Changes in relative difference in carbon density (*CRD*, %) in each mineral soil layer grouped in age classes of 5 years and *CRD* classes of 20% for all species considered.

The average mineral soil C density in pastures and afforested stands, grouped in age classes of 10 years and for the three mineral soil layers studied, are shown in Fig. 2.4. For direct comparison of the C densities in each soil depth, the value of each soil layer was divided by the corresponding depth (Mg ha^{-1} cm^{-1}). The average SOC densities in pasture sub-plots were constant, since there were no significant changes in carbon densities over time for any of the species studied ($p<0.001$). Changes in *CRD* and *CAD* were therefore only due to changes in SOC densities in forest subplots, because carbon remained constant for a given soil layer in pasture land. There were no significant differences in the distribution of SOC across soil depth either between species or ages considered ($p<0.001$). Nevertheless, there were significant differences in *P. radiata* stands in the upper mineral soil layers, as previously reported.

On the other hand, the mean changes in *CAD* and the 95% confidence levels in the 0-15 cm mineral soil (and in the litter layer) for each of the three species considered in this study throughout their respective rotations are shown in Fig. 2.2. For calculation of the average trends and confidence levels, the nonparametric procedure fitting described above was applied. This type of representation enables consideration of the variability in the data. Significant SOC losses were detected in the first 5-10 years after afforestation in *P. radiata* stands. The average losses amounted to -10.1 Mg ha^{-1} (for 95% of confidence level, between -7.1 to -13.0), which constituted an average loss of -24.1% of the initial SOC (for 95% of confidence level, between 18.8-29.4%). There were then large gains in soil C, coinciding with significant accumulation of litter, reflecting a change in the environmental equilibrium between decomposition and production. The average compensation age (the time at which the initial SOC content is recovered) was 20 yr (for 95% of confidence level, the data ranged from 14 to 25 yr) and progressive gains occurred thereafter.

Thus, at the end of the first rotation the mean gain in SOC in these mineral soils was +9.2 Mg ha^{-1} (for 95% of confidence level, between +3.8 and +15.0 Mg ha^{-1}) relative to the pastures where they were established.

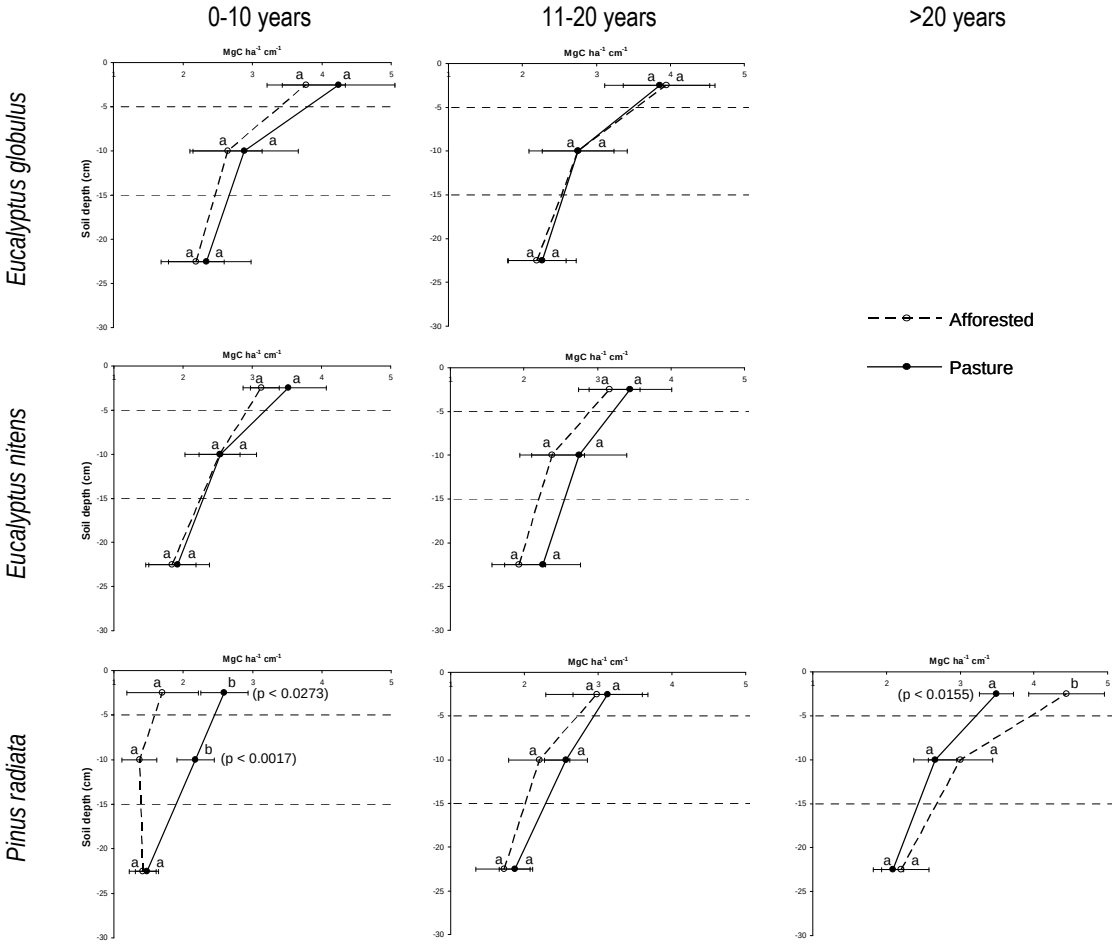

Figure 2.4. Changes in soil carbon density with time since afforestation for each soil depth. (n=40 for each species).

In the eucalypt stands, the mean changes in SOC were rather different from those of *P. radiata*. Despite the high variability observed, a general trend was distinguished. The initial SOC losses started later than in the *P. radiata* plantations (Fig. 2.2), and although they were of lower intensity in terms of *CRD* (Table 2.2), the average *CAD* values were similar. In the *E. nitens* stands, in which the litter accumulation started earlier, the SOC levels remained rather close to the initial contents throughout the whole rotation, and losses were slightly lower (-7.2 Mg ha^{-1}, values ranged from +0.1 to -15.2 Mg ha^{-1}). In *E. globulus*, a slight recovery appeared to occur at the end of the rotation, but the maximum losses for this species amounted to -9.8 Mg ha^{-1} at age 7 years (+2.0 to -23.8 Mg ha^{-1}), leading to average losses of -9.2% (+0.7% and -19.1%) of the initial SOC.

Levels of C accumulation in the soil throughout the first rotation were calculated considering the two soil pools jointly, litter layer and mineral soil (Fig. 2.5). Net gains in C were observed from the beginning of the rotation in the soils under *E. nitens*, as a result of the large contribution of the organic layer in these stands. In contrast, significant gains were observed in *E. globulus* and *P. radiata* 8-9 yr after afforestation (Fig. 2.5). In both *Eucalyptus* stands the soil C density stabilised

15 yr after afforestation, whereas in the pine stands the soil C accumulation was still increasing after the usual rotation length (30 yr). Root biomass (including stump) was not considered in this study.

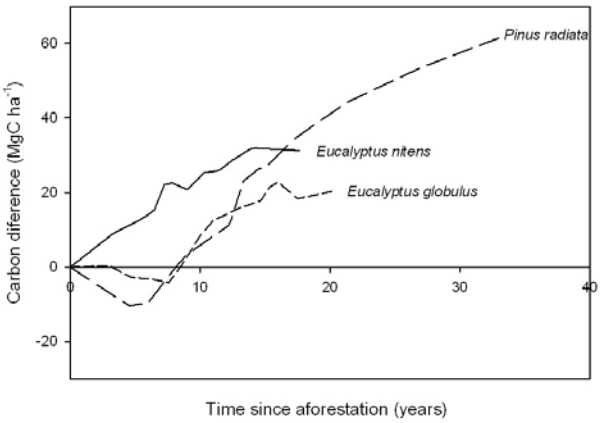

Figure 2.5. Changes in total soil C (litter layer plus 0-15 cm mineral soil) throughout the rotation for all three species considered.

To evaluate the effects of site characteristics on SOC dynamics after land use change, the plots were classified into two groups, according to whether the *CAD* was above or below the average non-parametric level, as shown in Fig. 2.2. No differences in site index were revealed by the unbalanced variance analysis technique, although the greatest gains were systematically observed in stands with the highest site index values (Table 2.4). Nonetheless, there were significant differences in the initial carbon content between the upper and lower part of the curve for plots of both *Eucalyptus* species, in which the greatest losses in the mineral soil coincided with the highest initial C density, although this effect was not significant for *P. radiata* (Table 2.4). Although soil texture is known to affect SOC dynamics, there were no significant differences between the upper and lower part of the *CAD* curve for this parameter, nor any apparent trend, probably because of the high homogeneity among plots.

Table 2.4. Average values (and standard deviations) for soil texture, site index and carbon concentration for the upper and lower part of the carbon density accumulation curve (Fig. 2.2). Different letters indicate significant differences ($p<0.01$)

	Position	Texture		Site	Initial carbon concentration (%)		
		Clay (%)	Sand (%)	Index (m)	0-5 cm	5-15 cm	15-30 cm
E. globulus	Upper	15.37 (5.3)	42.77 (14.2)	23.6 (7.1)	4.95 (2.2)a	2.92 (1.5)a	2.53 (1.2)a
	Lower	12.11 (5.3)	50.92 (11.1)	23.1 (6.0)	6.67 (2.1)b	5.08 (1.7)b	4.09 (1.4)b
E. nitens	Upper	11.74 (5.7)	44.82 (12.7)	16.1 (4.2)	5.51 (2.8)	3.58 (1.4)a	2.88 (1.2)a
	Lower	12.56 (6.7)	44.98 (18.6)	14.4 (4.6)	6.86 (2.3)	5.62 (2.3)b	4.31 (2.0)b
P. radiata	Upper	8.48 (3.8)	57.19 (10.5)	25.4 (4.5)	3.95 (1.0)	3.13 (0.8)	2.43 (0.5)
	Lower	10.77 (5.4)	50.40 (13.7)	24.3 (4.3)	4.12 (1.1)	3.35 (0.5)	2.37 (0.6)

On the other hand, the average C/N ratio in the pasture mineral soil was 12.4 (2.1, Std. dev.) and, as expected, this did not change with respect to the age of the paired afforested stands (data not shown). The C/N ratio in the *P. radiata* stands increased with age (R^2= 0.3; p<0.05) reaching an average value of 18.2 at the end of the rotation, which differed significantly from the values in the pasture soils (p <0.01). In contrast, the C/N ratio in the soils under eucalypts did not differ from those in the paired pastures.

2.3.4. Changes in C in the forest system throughout the first rotation after afforestation

The average changes in C (Mg ha^{-1} yr^{-1}) throughout the rotation, taking into account the aboveground tree biomass, the organic layer and the mineral for each of the three species are shown in Fig. 2.6. In order to analyse the effect of the rotation length on the C sink capacity, the total C amounts accumulated in aboveground tree biomass and the accumulation rates considering three possible rotation lengths for each tree species are shown in Table 2.5.

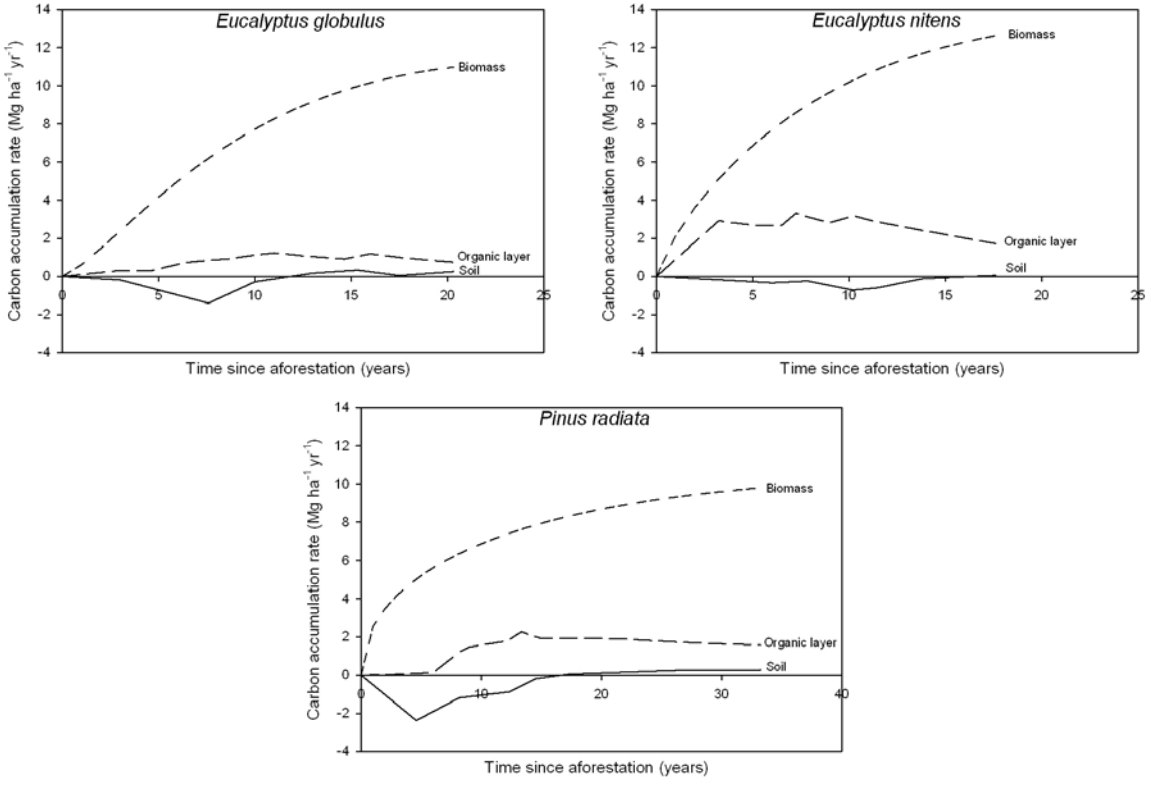

Figure 2.6. Changes in the C density accumulation rate (Mg ha^{-1} yr^{-1}) in aboveground biomass, organic layer and mineral soil (up to 15 cm depth) over time (n= 40 for each of the three species).

The effectiveness of C sequestration followed the order: *E. nitens* > *E. globulus* ≥ *P. radiata*. The components accumulated C according to the following order: above ground biomass (94-96%) > litter layer (3.4-10.8%) > 0-15 cm upper soil mineral (0.17-0.96%). At the end of each rotation the C accumulated followed the order: *P. radiata* > *E. nitens* > *E. globulus.*

Table 2.5. C accumulation in aboveground biomass, litter layer and mineral soil (0-15 cm) considering three possible rotation lengths.

	C pool	*E. globulus*			*E. nitens*			*P. radiata*	
		10 yr_1	15 yr_2	20 yr_3	10 yr_1	15 yr_2	20 yr_3	30 $yr_{1,2}$	35 yr_3
C accumulation rate	Biomass	7.73	9.87	10.93	10.19	12.04	13.00	9.61	9.89
(Mg ha^{-1} yr^{-1})	Litter layer	1.18	0.90	0.87	2.92	2.17	1.51	1.62	1.49
	Mineral soil	-0.24	0.17	0.18	-0.56	-0.05	0.05	0.28	0.27
	Total	8.67	10.94	11.98	12.55	14.16	14.56	11.51	11.65
C accumulation	Biomass	77.3	148.0	218.6	101.9	180.6	260.0	288.3	346.1
(Mg ha^{-1})	Litter layer	11.8	13.5	17.3	29.2	32.5	30.2	48.5	52.3
	Mineral soil	-2.4	2.6	3.6	-5.6	-0.7	1.0	8.4	9.5
	Total	86.7	164.1	239.5	125.5	212.4	291.2	345.2	407.9

$_{1, 2}$ Most common rotation lengths in afforested agricultural land in the region. $_3$ Long rotation

In all three species the ecosystem C accumulation rates kept increasing even in the longest rotations (Table 2.5). The most important effect was found in the eucalypts stands in which the C sink rate increased by 2.0-3.3 Mg C ha^{-1} yr^{-1}. Prolongation of the rotation length in these species also resulted in net C gains in the mineral soil.

2.4. Discussion

2.4.1. Experimental design

The present study involved 120 paired plots (40 per tree species) in a geographical area of 7000 km^2, which is rather homogeneous from the point of view of climate and soil properties. The experimental design, based on well-replicated chronosequences combined with paired plots, enabled most of the variability and local conditions to be controlled, and average regional trends in the C dynamics for the three most common tree species used in afforestations in northern Spain to be determined. Despite the rather homogeneous environmental conditions, the variability in the C dynamics after afforestation was high, especially in the *Eucalyptus* chronosequences. Such high variability, which has also already reported in other studies at landscape scale (Johnston *et al.*, 1996; Turner & Lambert, 2000; Conant *et al.*, 2003; Poeplau *et al.*, 2011), emphasizes the risk of reaching wrong conclusions about SOC dynamics when the experimental design does not take into account most of the variability.

2.4.2. Changes in C in aboveground biomass and litter

The growth rates of the fast-growing tree species studied differed widely. The mean annual increments were higher than the productivity recorded for the plantations established on former forest soils in the region (Balboa-Murias *et al.*, 2006). The enhanced growth is attributed to the greater depth and availability of nutrients and water in the pasture soils. Trees growth was rapid despite the poor nutritional conditions of the plantations (Merino *et al.*, 2003; Zas & Serrada, 2003), possibly because of an efficient use of available nutrients in the species considered (Judd *et al.*, 1996).

At the end of the rotation, the litter constituted between 3 and 11% of the forest system C pool. The C sequestration rates in the organic layer (0.9-2.9 Mg C ha^{-1} yr^{-1}) were considerably higher that those reported for Central Europe and Scandinavian Countries ((Vesterdal *et al.*, 2008; Berg *et al.*, 2009) lower than 0.5 Mg C ha^{-1} yr^{-1}) and lower than in tropical forests (Ostertag *et al.* (2008), 5 Mg C ha^{-1} yr^{-1}).

The different litter accumulation rates associated with the three species only partly correspond to the tree growth rates. Thus, the high litter accumulation in *E. nitens* stands was possibly associated with the particularly high growth rate of this species (Fig. 2.1). However, litter accumulation was lower in the *E. globulus* stands than in *P. radiata* stands, despite the greater increase in biomass. Moreover, within the same species, the relationships between the changes in tree biomass and litter were weak, indicating that litter accumulation was also determined by other factors, such as decomposition rates or changes in ground cover.

Thus, the lower litter accumulation in *E. globulus* may be due to the faster decomposition of eucalypt litter than of *P. radiata* litter, as recorded in the region (Alvarez *et al.*, 2008) and elsewhere (Paul & Polglase, 2004; Lemma *et al.*, 2007; Huang *et al.*, 2011), and attributable to differences in the chemical composition of the litter. Litter decomposition in eucalyptus may also have been enhanced by favourable microclimatic conditions. On one hand, *E. globulus* stands are located at lower altitudes (lower than 300 m.a.s.l.), with less extreme temperatures. In addition, light transmission is higher in *Eucalyptus* than in pine stands, because of the arrangement of the leaves (González-Hernández *et al.*, 1998), and probably derivates in higher soil temperatures and more favourable moisture levels (Martius *et al.*, 2004b). This may have promoted decomposer and macrofauna activities and thus higher decomposition rates (Martius *et al.*, 2004a).

With the exception of *E. nitens* stands, in which litter accumulation occurred very early on, in *E. globulus* and *P. radiata* stands development of the litter layer coincided with the canopy closure (around 5 yr after establishment). This implied an increased input of litter, as well a lower mineralization rate as a consequence of shading.

In addition to these factors, ground vegetation was rather abundant in these young plantations (3-8 Mg ha^{-2}; Omil *et al.* (2007) and Carneiro *et al.* (2009), but decreased steadily with the increased shading throughout stand development (Fernández-Núñez *et al.,* 2010). Thus, canopy closure may have resulted in a sharp decrease in ground vegetation root turnover. This may have

been greater under pine because of the greater degree of shade provided by pines than by eucalypts (despite the usual tree density in the latter plantations). Thus, Omil *et al.* (2007) recorded a decrease of 50% in the ground vegetation during the first 10 yr after the establishment of a *P. radiata* plantation. On the other hand, the death of ground vegetation as a result of shading in *P. radiata* stands contributes to the aboveground litter. Higher litter production in association with the ground vegetation has also been reported elsewhere (Stendahl *et al.*, 2010).

2.4.3. Changes in C in mineral soil

The plantations under study replaced pastures growing on soils with rather high SOC contents (74.8 Mg ha^{-1} to a depth of 30 cm, 27.7 Mg ha^{-1} Std. dev.), intermediate between croplands and natural forest soils in the region (Leirós *et al.*, 2000; Merino *et al.*, 2004). The high SOC contents are due to long use under low intensive management based on low intensity tillage and addition of slurry, lime and fertilizers, which enhance grass production and therefore root turnover. Other researchers have indicated the potential of grasslands to sequester SOC, in some cases at levels comparable to those in forests (Corre *et al.*, 1999; Jackson *et al.*, 2002).

The data obtained in this study revealed important losses of SOC in the upper mineral soils during the first 10 years after afforestation, and subsequent gains, which may lead to recovery of the initial levels after 20 years. The maximum SOC losses in the first 10 years after afforestation in the upper 0-15 cm mineral soil layer were dependent on the tree species and constituted 4-38% of the initial C contents. Such high losses are common in afforested grassland soils under humid temperate climates (Kirschbaum *et al.*, 2008) and occur as a consequence of mineralization of the high content of the labile fraction in these soils, which was not compensated by decreased litter inputs from grass litter (Don *et al.*, 2009; Huang *et al.*, 2011). In some cases, they are caused by soil perturbation during site preparation work (Turner & Lambert, 2000; Maillard *et al.*, 2010). Anyway, soil perturbation was low in the present study since the site preparation during forest establishment was of low intensity.

In addition to the favourable climatic conditions, the high SOC losses in these soils may also be caused by certain soil properties that favour the rapid turnover of SOM. The soils under study contained low percentages of clay (less than 20%; the texture is sandy loam), which is dominated by minerals of low surface reactivity (kaolinite and oxides of Al and Fe). Therefore, the capacity of these soils to stabilize C in mineral-associated forms and in fine pores may be limited. Nevertheless, chemical stabilization due the saturation of Al and Fe in the cation exchange complex (Hobbie *et al.*, 2007) should also be considered. Different studies have revealed that SOM turnover is usually rapid in the uppermost soil horizons, but takes longer as the soil depth increases (e.g. von Lützow *et al.*, 2006). It is assumed that SOM stabilization mechanisms (physical inaccessibility to microorganism and enzymes or interaction with mineral surfaces and metal ions) are less active in the uppermost layers of mineral soil than in deeper soil horizons (e.g. von Lützow *et al.*, 2006). A recent study of the same soils revealed that the SOC degradation does

not only affect the more labile organic SOM compounds, but also complex and recalcitrant C compounds (Chapter V).

Similar patterns to those described above have been described in other studies (Table 2.6). These studies were selected to represent the same land use change as described in the present study, and to provide information enabling estimates in the short and long-term and at compensation age. In the short term (<20 yr), the average losses were similar to those observed in the present study. The average C compensation ages recorded in the present study, between 10 and 25 years, were within the most common range reported in the relevant literature (Table 2.6), although much longer compensation ages (80 yr) have been simulated in colder climates than described here. Moreover, greater (although highly variable) long-term gains than those observed here have been reported, although the time frame considered was longer than in the present study (Table 2.6).

The present results show that the subsequent gains in SOC took place after canopy closure in the stands. Significant C gains were only recorded in the 15 cm upper mineral soil layer. However, it is known that root turnover from trees can incorporate organic matter deeper than 30 cm (Brown & Lugo, 1990; Trumbore *et al.*, 1995; Jackson *et al.*, 1996; Jobbágy & Jackson, 2000), although C accumulation in root biomass was not taken into account here. The subsoil horizons were very variable (A2, AB, B), and the SOC contents were different, which may have prevented identification of any clear trends.

Furthermore, within the same species, the SOC gains were slightly higher in the stands with higher site indexes, which reflects the influence of the greater biomass production on litter production. Nevertheless, the effect of the site index was not able to be evaluated accurately, since site index was rather high in most cases. This aspect is of interest, as climate change is expected to change site index, and therefore net primary production, worldwide, and the effect on changes in SOC required further investigation. Soil texture is one of the most important factors controlling SOC dynamics, and SOC increases with clay content in afforested soils (Mendham *et al.*, 2003). The present study, however, did not identify any changes in the SOC dynamics attributable to soil texture, probably because the soils were rather homogeneous as regards this parameter.

Table 2.6. Changes in upper mineral soil carbon after land use change from agriculture to pasture to forest reported in several reference studies. For the values obtained in the present study: short term (S.T.) and long term (L.T.) effects show the average and the range of values provided by LOESS analysis. The range of compensation age (*Pinus radiata*) and the *CRD* compensation age (COMP.) are also shown.

Source	Species	Origen	Ref. land use	Approach	Mineral soil dept (cm)	S. T. EFFECT		COMP.	L. T. EFFECT	
						Age (yr)	Effect	(yr)	Age (yr)	Effect
Zak *et al*. (1990)	*Quercus ellipsoidalis*	A	S	CH	0-10	8-9	-31%	20	40	+35%
Johnston *et al*. (1996)	Five forest types	A	S	CH	0-10	5-10	-10%	20	40	+40%
Giddens *et al*. (1997)	*Pinus radiata*	P	PL	PP	0-10	-	-	16-24(2)	-	-
Bashkin & Binkley (1998)	*Eucalyptus saligna*	A	PL	PP	0-10	-	-	10-13	-	-
Richter *et al*. (1999)	*Pinus taeda*	A	PL	LT	0-7.5	6-10	-18%	16-18	35	+22%
Ross *et al*. (1999)	*Pinus radiata*	P	PL	PP	0-10	19	-13%	-	-	-
Jug *et al*. (1999)(1)	*Populus* spp; *Salix*	A, P	PL	LT	0-5	10	+50%	-	-	-
	Populus spp; *Salix*	A,P	PL	LT	0-5	7	+17%	-	-	-
Turner & Lambert (2000)	*Eucalyptus grandis*	P	PL	PP, CH	0-10	15	-40%	-	35	-40%
Vesterdal *et al*. (2002)	*Quercus robur*; *Picea*	P	PL	CH	0-5	5-10	-45%	-	29	-25%
Hooker & Compton	*Pinus strobus*	P	S	CH	0-20	-	-	-	115	0%
Ussiri *et al*. (2006)	*Casuarina* spp.	P	PL	PP	0-10	10	-16%	-	-	-
	Robinia pseudoacacea	P	PL	PP	0-10	10	-11%	-	-	-
Thuille & Schulze (2006)	*Picea abies*	P	PL	CH	0-50	15-60	-30%	80(3)	93-112	+0.24-+0.34(6)
Morris *et al*. (2007)	Coniferous spp.	A	PL	PP	100	-	-	-	65	+24.7%
	Deciduous spp.	A	PL	PP	100	-	-	-	65	+35.6%
This study	*Eucalyptus globulus*	P	PL	PP, CH	0-15	8	-22.2% (-52.0%-+2.7%)	12(4) (-15.7%-+21.7%)	21	+10.8% (+2.0%-+24.2%)
	Eucalyptus nitens	P	PL	PP, CH	0-15	11	-16.7% (-34.7%-+0.2%)	16(4) (-15.5%-+18.8%)	18	+2.2% (-15.6%-+18.8%)
	Pinus radiata	P	PL	PP, CH	0-15	5-13	-26.0% (-34.4%--17.0%)	17 (14-25)(5) (-21.4%-+27.9%)	34	+23.5% (+10.6%-+37.4%)

A, agriculture; P, pasture; S, secondary succession, PL, plantation; CH, chronosequence; PP, paired plots; LT, long term study.
(1) Fertilized short rotation plantations. (2) For 60% of the studied sites. (3) Obtained from simulation model. (4) Compensation age for the average value; (5) Compensation age for the 95% confidence intervals. (6) Mg C ha^{-1} yr^{-1}.

2.4.4. Influence of species

The results of the present study revealed important differences in the SOC dynamics following afforestation, attributable to the tree species and the associated vegetation. Although SOC losses were always recorded after afforestation, in the soils under the two eucalypt species, losses were generally lower and the periods of loss were shorter. In the *P. radiata* stands there was clear net gain of SOC from 25 years onwards, because of the longer rotation. In both eucalypt stands, the compensation ages were close to the end of the rotation, which implies no net gains prior to cutting in most cases. Longer rotation in eucalypts may lead to positive SOC gains.

Moreover, the variability in SOC in the earlier period (0-10 yr) was much higher in both *Eucalyptus* stands than in *P. radiata* (Fig. 2.2). This was probably due to the death of weeds and herbaceous species in the forest subplot because of shading, which leads to loss of SOC in all plots, independently of the initial conditions. In the *P. radiata* plots (Table 2.4) there were no significant differences between the upper and lower part of the *CAD* curve (Fig. 2.2).

The different patterns in the SOC dynamics may be determined by the different SOM dynamics and litter turnover for the three tree species studied. Thus, it is possible that the rapid litter turnover in both types of eucalyptus stands prevented SOC losses in the first years after afforestation, as also suggested by Vesterdal *et al.* (2008), and Huang *et al.* (2011). However, the differences in SOC may also be due to the different ground vegetation development in eucalyptus and pine stands, which affects the SOC via different mechanisms (Lugo & Brown, 1993; Silver *et al.*, 2004). In the pine plantations, the wider crown intercepting solar radiation exerts a negative influence on the ground vegetation (very large decreases in the ground vegetation occur from the 5th year (Omil *et al.*, 2007)) and probably also negatively affects litter decomposition. Thus, the higher losses of SOC observed in the young pine plantations may be due to the lower transfer of organic C to the mineral soil, as a consequence of the lower litter inputs from the ground vegetation and the lower decomposition rate of the litter.

The opposite occurs in the eucalyptus plantations, in which the higher crown light-transmission favours higher ground vegetation cover throughout the whole rotation, and with a high presence of grass species (González-Hernández *et al.*, 1998; Silva-Pando *et al.*, 2002). The different ground vegetation cover probably determined the amounts and the type of litter (aerial, root) in these plantations. Thus, the presence of grass in these young plantations resulted in greater belowground C inputs such as root biomass turnover and root exudates (Jones *et al.*, 2009). Grass material is incorporated more rapidly than litter layer material into soil organic matter (Andrade *et al.*, 2008; Laungani & Knops, 2009). The lower losses of SOC in eucalypt soils may therefore be due to the higher inputs of litter from grasses in the ground vegetation, thus compensating for the initial losses of SOC following afforestation. Similar mechanisms has also been suggested by Lemma *et al.* (2006) and Huang *et al.* (2011) to explain the greater SOC gains in soils afforested with *Pinus patula* and *E. nitens*, respectively.

Moreover, the C/N ratios in the mineral soil layer of the mature afforested soils under pines increased throughout the rotation. This effect has also been reported in other studies (Smethurst & Sadanandan Nambiar, 1995; Giddens *et al.*, 1997; Jug *et al.*, 1999; Ussiri *et al.*, 2006), and is probably due the increased influence of forest litter on SOM quality throughout the rotation. This would reflect a shift from organic input dominated by grass litter, to forest litter containing greater amounts of recalcitrant biopolymers (resins, waxes, suberin and cutin-derived compounds (Chefetz *et al.*, 2002; Otto & Simpson, 2006)). The production of recalcitrant compounds from this type of litter and their release to the mineral soil may therefore explain the higher C/N ratio in the soils under mature pine plantations. The higher C/N ratio in the afforested soils may also be due to a lower presence of legumes in the understory vegetation (Corbeels *et al.*, 2002) and to higher N immobilization in trees.

However, the C/N ratio was not higher in the eucalyptus stands, possibly because of the presence of more grass in the underground vegetation in these plantations. Soil analyses revealed the presence of more carbohydrates in the SOM in these mature stands, reflecting different sources of litter (possibly due to the input of root litter and root exudates) relative to the mineral soil under pine, in which more recalcitrant compounds were identified (Chapter V).

2.4.5. Increasing the C sink capacity by tree species selection and management

The data obtained in the present study show that of the species studied, *E. nitens* has the highest C sink capacity, followed by *E. globulus* and, very closely by *P. radiata*. The mean rates of C sequestration (biomass and soil) estimated in this study for the most common rotations (Table 2.5) ranged between 8.7 and 12.6 Mg C ha^{-1} yr^{-1} (average value for the three species, 10.9 Mg C ha^{-1} yr^{-1}). Considering that the afforested area in northern Spain using these three species can be estimated as 135000 ha for the period 1994-2006 (MAPA, 2006), afforestation would have resulted in a sink of 1.2-1.7 Tg C yr^{-1} (average 1.5 Tg C yr^{-1}), with respect to the Spanish CO_2 emissions (101 Tg C in the year 2009, (MMAMRM, 2010)). This indicates the significant contribution of afforestation to the mitigation of CO_2 emissions, and also shows that selection of the tree species is a major factor influencing the C sink capacity.

Prolongation of the rotation by 10 and 5 years for *Eucalyptus* and *P. radiata* respectively resulted in a C sequestration rate ranging between 10.9 and 14.2 Mg C ha^{-1} yr^{-1} (average value for the three species, 12.7 Mg C ha^{-1} yr^{-1}, Table 2.5), which implies a sink of 1.6-2.3 Tg C yr^{-1} (average 1.7 Tg C ha^{-1} yr^{-1}). These and previous results (Balboa-Murias *et al.*, 2006; Diaz-Balteiro *et al.*, 2009) show that in order to maximize the C sink capacity, plantations should be managed according to the optimal harvesting schedules for these species. In the present study, the data show that the C sink capacity of these plantations can be increased greatly by prolonging the rotation age.

The selection of tree species and the harvest scheduling may also favour C gains in the soil. The contribution of the soil (litter plus mineral soil) to the overall C sequestration ranged from 8 to

18% (in average, 15%), which is similar to the inputs reported by De Vries *et al.* (2006) and Woodbury *et al.* (2006), in Europe and United States, respectively and lower than those reported by Liski *et al.* (2002).

Nevertheless, since these intensively managed plantations are harvested repeatedly, the equilibrium will probably be determined by the specific management of each species, particularly by the intensity of the harvesting and disturbance after cutting. Thus, unlike in colder climates (Berg *et al.*, 2009), in these intensively managed forests the C contained in the litter cannot be considered as a medium-term store of stored C because it may be rapidly lost after clear cutting or site preparation. This is particularly important in the two *Eucalyptus* plantations, in which management in short rotations may lead to continuous loss of SOC. On the contrary, since plantations of *E. globulus* are coppiced (i.e. the root system is kept without any site preparation), the subsequent rotation will grow faster and SOC will probably continue to increase.

Although the site preparation techniques carried out in the region (ripping or holing), do not involve important soil disturbance, logging residues are often removed from the site for energy production. This reduces litter input and nutrient returns (Merino *et al.*, 2005), which may reduce the C sink capacity of both biomass and soil (Maillard *et al.*, 2010). Thus, net losses of soil C have been recorded in soils subjected to high degree of disturbance for site preparation (Pérez-Batallón *et al.*, 2001).

On the other hand, the main limiting nutrients in these plantations are P and Mg. Improvement of the nutritional status of the plantation not only implies higher biomass growth rates, but also favours accumulation of SOC (Turner *et al.*, 2005). The application of charcoal-containing wood ash, a by-product of biomass power plants, is increasingly carried out in the area. The application of wood ash replenishes nutrients and enhances timber production (e.g. Solla-Gullon *et al.*, 2008; Pérez-Cruzado *et al.*, 2011). Moreover, charcoal is relatively recalcitrant and can therefore act as a long-term sink for atmospheric CO_2 (e.g. Krull *et al.*, 2006).

2.5. Conclusions

In this study an intensive sampling scheme was used to assess the C sink capacity of forest stands of the three species most commonly used in afforestation programmes in northern Spain. The high spatial variability in the different compartments illustrates the risk of reaching wrong conclusions about SOC dynamics when the experimental design does not cover most of the variability.

The humid temperate climate resulted in C accumulation rates as high as 9-14 Mg C ha^{-1} yr^{-1} in the first 20 yr, depending on the species and the rotation length. The results of the study show how selection of the tree species is a major factor influencing the post afforestation C sink capacity, affecting the amounts of C accumulated in both biomass and soil.

The role of the tree species is particularly important during the first years after afforestation when the litter input from herbaceous vegetation may compensate for losses of SOC. The patterns of SOC dynamics differed greatly in relation to the different tree species used in the afforestation, and were determined by transfer of C to the soil via the roots of the ground vegetation and the turnover rate of the litter. Both of these sources were lower in the pine plantations than in the eucalyptus plantations, which may explain the higher SOC during the first years after afforestation.

The humid temperate climate, along with the lack of physically protected SOM (sandy loam texture of the soils) favoured important losses of SOC in the uppermost mineral soils during the first years.

The study provides accurate information on the success of these afforestation programmes as regards CO_2 mitigation. To enhance the C sink capacity, plantations should be managed according to optimal harvesting schedules for the species. Elongation of the rotation length led to larger C sink capacities in all three species. This is especially important in such intensively managed plantations, in which harvesting in short rotations may lead to continuous loss of SOC.

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Chapter III

Improvement in accuracy of aboveground biomass estimation in Eucalyptus nitens *plantations: effect of bole sampling intensity and explanatory variables*

3. Improvement in accuracy of aboveground biomass estimation in *Eucalyptus nitens* plantations: effect of bole sampling intensity and explanatory variables

Abstract

Two sets of aboveground biomass equations were fitted for stem only and stem plus crown predictive variables in *Eucalyptus nitens* plantations in Northern Spain. A sample of 40 trees was chosen after a complete study of variation in tree height and diameter in the region. The trees were felled and the biomass was divided into the following components: wood, bark, thick branches, thin branches, twigs, leaves and dead branches along the stem. Bole biomass was estimated by systematic subsampling of one 5 cm-thick disk every 0.5 m. Such intensive sampling enabled determination of the effect of subsampling intensity on accuracy and bias of wood estimation, considering two ratio-type estimators: stem weight to dry matter, determined by the complete weighing (*CW*) method (i.e. of the fresh weight of the entire stem) and volume to dry matter, determined by the partial weighing (*PW*) method. The changes in moisture content and basic density along the stem explained the serious risk of dry mass or weight overestimation when a systematic subsample is considered. The average basic density was usually found at a relative height of 30-35% along the stem. The default choice of the bottom disk or log as the first section resulted in overestimations for the *CW* method and underestimations for the *PW* one. The biomass equations were fitted by seemingly unrelated regression, with corrections for heteroscedasticity carried out by weighted fitting. Diameter at breast height was the best explanatory variable, and the inclusion of height did not improve the accuracy, except for wood. The inclusion of crown variables improved the predictive ability for crown fractions, increasing the accuracy for estimating thick branches (by 10.8%), twigs (by 19.1%) and leaves (by 17.3%). The biomass of each fraction decreased in the following order: wood > bark > thick branches > dead branches along the stem > leaves > thin branches > twigs. The changes in these percentages with diameter class and the predictive ability of the fitted equations were also studied.

Keywords: *Eucalyptus nitens*, biomass, ratio type estimators, wood basic density, wood moisture; crown variables.

3.1. Introduction

Destructive sampling and subsequent regression analysis is the most common method used to estimate tree biomass (Parresol, 1999). Biomass estimation at tree level is a necessary first stage in estimating stand biomass, and the main sources of error in this process are: (i) selection of trees for sampling; (ii) measurement of independent and dependent variables in sampling trees; (iii) choice of a suitable form of the allometric relationship and values for any adjustable parameters in the equation; (iv) field measurement of the independent variables in the objective population, and (v) application of allometric equations to objective populations for individual tree biomass estimation and summation to obtain stand estimates (Cunia, 1987; Ketterings *et al.*, 2001). Each of these steps has an associated error that must be minimized; the errors involved in step (ii) are the least well studied (Satoo & Madgwick, 1982; Cunia, 1987; Parresol, 1999; Ketterings *et al.*, 2001).

The errors in the assessment of sample tree dependent variables are strongly influenced by the procedure (Cunia, 1987): (i) subsampling selection, (ii) fresh and dry weight estimation, and (iii) subsampling intensity. With small trees, fresh and dry weighing of the entire tree is not time consuming nor expensive and is therefore recommended (Parresol, 2001). However, direct measurement becomes more expensive as tree size increases, and subsampling becomes inevitable (Satoo & Madgwick, 1982; Parresol, 1999). Fresh weight can be measured directly or estimated by several methods. One of the most commonly used methods of determining the fresh weight of trees and estimating the dry weight is to use ratio-type estimators (Briggs *et al.*, 1987), in which the relationships between dry/fresh weight or dry weight/fresh volume are assessed in a sample and applied to the rest of the tree for dry weight estimation. The main advantage of these methods is the simplicity of application and determination, although it is well known that ratio estimators are biased (Cunia, 1979; Valentine *et al.*, 1984).

Some methods provide unbiased, efficient estimations, such as randomized-branch sampling (*RBS*) and importance sampling (*IS*) methods, which use auxiliary information to select elements in the sample to reduce the variance of the estimator (Valentine *et al.*, 1984; Parresol, 1999). *RBS* is a type of multi-stage probability sampling, which is used to select a path so that resultant segments of the path comprise a probability sampling of the entire tree. *IS* is a continuous analog involving sampling discrete units with probability proportional to size (Gregoire *et al.*, 1995). These methods are of interest for estimating fractions such as branches or foliage, although in practice they are time-consuming and difficult to apply.

To apply ratio type estimators, stems can be weighed and disks removed to determine moisture content (by the complete fresh weighing, *CW* method) or volume can be estimated and short sample logs weighed to obtain volume to mass conversion factors (by the partial weighing, *PW* method). Subsampling across the bole can be done by random stratified sampling, as carried out by Briggs *et al.* (1987), although most researchers use a fixed number of sections across the stem, with the position chosen systematically (i.e. (Saint-André *et al.*, 2005)), randomly, or with a

probability proportional to a given dimension. This was the case for Kleinn and Pelz, (1987), who chose disks with a probability of selection proportional to estimated volume. On the other hand, the *PW* method is preferred for large trees in sites with difficult access, as fresh weighing of the whole stem is quite laborious and time consuming (Snowdon *et al.*, 2000). In the *CW* method, the distribution of moisture along the stem is the main source of error for dry weight estimation, whereas in the *PW* method, it is the variation in basic density along the bole height that affects that error. In both cases, sampling intensity and distribution should guarantee a suitable description of the variability in moisture content and specific density.

Diameter at breast height (*d*) and total height (*h*) are the most common independent variables used in biomass regression, because of their ease of measurement and predictive capacity (Parresol, 1999; Snowdon *et al.*, 2000). However, because of the current increasing interest in obtaining accurate predictions of crown fractions for bioenergy, nutrient stability and silvicultural or ecological studies, there is a corresponding increasing interest in crown biomass modelling. Some authors have observed that the use of crown variables as explanatory variables improves the accuracy of biomass equations (Satoo & Madgwick, 1982; António *et al.*, 2007). In biomass studies in which high precision is required for crown fractions, and destructive sampling cannot be applied, highly accurate models are required.

The objectives of the present study were: (i) to obtain biomass estimation tools for a fast growing species, *Eucalyptus nitens*, in northwestern Spain, considering the most complete set of aboveground components; (ii) to evaluate the bias and accuracy of wood biomass estimation for different intensities of systematic subsampling across the stem and two ratio-type estimators (dry/fresh weight and dry mass/fresh volume), (iii) to evaluate the increased accuracy derived from the inclusion of crown variables in the estimation of individual tree biomass components, and (iv) to evaluate the ability of the proposed equations to estimate the proportion of each biomass component over total aboveground biomass, for a range of diameter classes.

3.2. Material and methods

3.2.1. Study site and trees sampled

This study was carried out in northwestern Spain, in an inland area located at elevations of 500 to 1000 m.a.s.l., with average precipitation of 900-1200 mm and average annual temperature of 12-13ºC (Martínez Cortizas & Pérez Alberti, 1999). Although frost occurrence limits planting of the most common *Eucalyptus* species in Spain (*Eucalyptus globulus* Labill.), *Eucalyptus nitens* (Deane & Maiden) Maiden was successfully introduced in the mid 1990s, providing yields of 15-50 m^3 ha^{-1} yr^{-1} (Pérez-Cruzado, 2009).

As the aim of the present study was to construct biomass models that are as representative as possible, sampling consisted of two phases: (1) study of the variability of the most commonly used independent variables in biomass equations at tree level (*d* and *h*, see below) across the distribution area, and (2) destructive sampling of trees covering the observed range (Parresol, 1999). For this purpose, 76 plots were established (see location in Fig. 3.1), covering the observed range of ages and site qualities, with a minimum plot size of 314 m^2, which is generally suitable for biomass estimation procedures in plantations (Satoo & Madgwick, 1982).

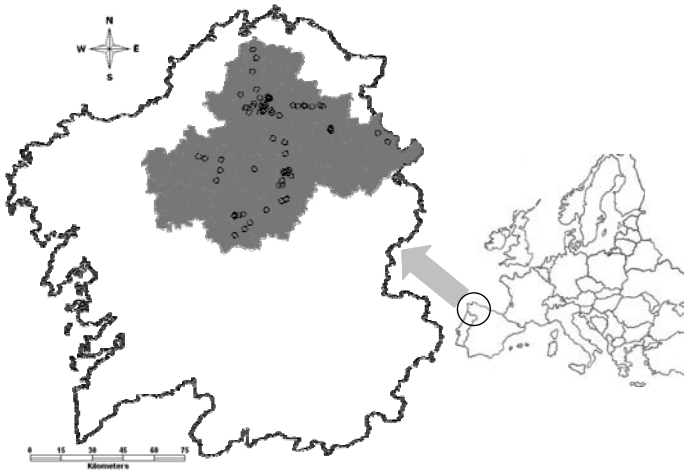

Figure 3.1. Location of the measured plots (dots) and the distribution of *Eucalyptus nitens* in north-western Spain (shaded area).

A sample size of 40 trees was chosen because of the low variability in site conditions and densities of plantations, most of which were established with the MacAlister provenance. The sampled trees were chosen in two steps, two trees per diameter and height class were first selected, and 16 additional trees were then chosen, considering the relative importance of each diameter class in the population. The aim of this procedure was to cover the full range of tree size, which is shown for height and diameter in Fig. 3.2. Trees were felled in 12 plots, in which the values of the quadratic mean diameter and *d* of the trees sampled was similar; undamaged, healthy trees that represented the dominant and codominant strata, were chosen. The average standard deviation and range of representative stand and single tree variables, for both the population and the sample are shown in Table 3.1. The variability in crown variables was similar to that observed in stem variables, unlike in other studies (Satoo & Madgwick, 1982).

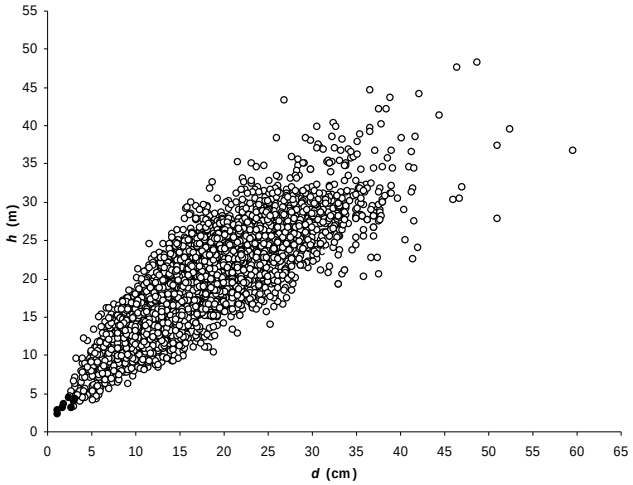

Figure 3.2. Height-diameter distribution of *Eucalyptus nitens* in an initial inventory in north-western Spain.

Table 3.1. Statistics for stand and single tree variables in the population (76 plots, 3864 trees) and the sample plots (12 plots, 40 trees).

		Stand variables			Individual tree variables	
		SI (m)	*N* (stems ha^{-1})	Age (yr)	*d* (cm)	*h* (m)
All plots	Average (Std. dev.)	15.3 (4.4)	1089 (280)	9.5 (4.2)	18.5 (7.5)	20.2 (6.4)
	Range	8.8 - 20.8	446 - 1560	2 - 18	1.0 - 59.6	2.2 - 48.3
Sample plots	Average (Std. dev.)	15.7 (2.7)	1101 (223)	10.2 (2.8)	19.5 (7.7)	19.3 (5.5)
	Range	9.8 - 18.9	446 - 1401	2 - 13	1.1 - 47.0	2.4 - 35.1

where *SI* is the site index (m at reference age of 6 years); *N* is stand density (stems ha^{-1}), *d* is diameter at breast height (cm), and *h* is the total height (m).

The following variables were measured in the sample trees while still standing: diameter at breast height (*d*, cm) and stump diameter at 0.15 m (d_{st}, cm), both measured in two perpendicular directions to the nearest mm; total height (*h*, m) and live crown base height, defined as the height of the first live branch insertion in the stem (h_{cb}, m), both measured to the nearest dm; crown diameter (d_c, m) measured in two perpendicular directions following the cardinal points to the nearest cm. Living crown length (h_c, m) was estimated as difference between total height (*h*) and live crown basis height (h_{cb}, m). Crown volume (v_c) was calculated from h_c and d_c by assimilating the crown shape to an ellipsoid [3.1]. Descriptive statistics for these variables are shown in Table 3.2.

$$v_c = \frac{4}{3}\pi \left(\frac{d_c}{2}\right)^2 \left(\frac{h_c}{2}\right) \qquad [3.1]$$

3.2.2. Ratio type estimators and subsampling

The felled trees were cut into 0.5 m logs to a small-end diameter of 7 cm. The logs were weighed fresh and a systematic subsample of one 5 cm-disk in the bottom part of each log was taken, also considering a further disk at the top of the stem. Sample disks were weighed fresh and transported to the laboratory in plastic bags. The over and under-bark diameters of the disks were measured in two directions and the bark and wood were then separated and weighed.

For each disk, the dry wood weight was measured after oven drying at 105ºC to constant weight and the ratio of the dry/fresh weight of the wood was determined. Only one composite sample per tree was considered for the bark. Fresh bark of all disks was weighed jointly, and dried to determine dry bark weight, thus enabling the ratio of dry/fresh weight of bark to be obtained for each tree.

Table 3.2. Descriptive statistics of sampled trees.

Variable	Average	Maximum	Minimum	St. Dev.
Independent variables				
d (cm)	20.84	41.55	3.95	10.04
d_{st} (cm)	25.63	52.40	6.60	12.13
h (m)	19.94	30.80	4.40	7.27
h_{cb} (m)	12.55	20.60	2.80	4.68
h_c (m)	7.39	19.80	1.20	4.21
d_c (cm)	3.50	8.55	1.25	1.66
v_c (m^3)	81.78	566.5	1.00	129.3
Dependent variables (kg $tree^{-1}$)				
W_l	10.73	48.85	0.28	12.94
W_t	4.15	23.33	0.18	5.10
W_{tb}	4.40	18.46	0.04	4.53
W_{Tb}	13.57	75.65	1.29	18.71
W_w	168.43	599.5	0	176.9
W_b	24.59	111.3	0	28.33
W_{db}	11.29	68.28	0.03	13.64
W_{tot}	237.2	838.2	2.54	248.1

Definitions of independent and dependent variables are given in section 3.2.2. W_{tot} refers to total aboveground biomass.

The dry weight of wood and of bark in each log was calculated from the average ratios calculated for the delimiting disks. The total wood (W_w, to a small-end diameter over bark of 7cm) and bark (W_b, evaluated till the threshold diameter considered for wood) dry biomass in each tree was calculated as sum of the biomass of each log.

Four biomass fractions were considered for the crown: thick branches (W_{Tb}, diameters over bark 2-7cm), which also include the tops of the boles, thin branches (W_{tb}, diameters over bark 0.5-2cm), twigs (W_t, diameter less than 0.5 cm) and leaves (W_l). Dead branches in the stem (W_{db}) is

also an important fraction in *E. nitens*. Crown biomass was first fractioned in the field into three groups: W_{Tb}, W_{db} and the sum of W_{tb}, Wt and W_l, and then weighed fresh, with a balance, to the nearest 10 g. A subsample of 10-15% of fresh weight of each fraction was taken to represent the top, medium and bottom part of the crown. These subsamples were weighed in the field, with scales, to the nearest 0.01 g.

The composite subsample of W_{tb}, W_t and W_l, was fractioned and weighed in the laboratory and the proportion of each fraction was determined to enable estimation of the fresh weight of each crown fraction. The dry weight of each fraction was then estimated from the dry/fresh weight ratios.

3.2.3. Methodologies for bole mass estimation

The information obtained enabled comparison of two methods of estimating bole mass or weight at a range of sampling intensities. The *CW* method consisted of determining the complete stem weight and estimating dry mass from disks. Disk subsampling intensity was modified considering a series of inter-disk distances which were multiples of 0.5. For each inter-disk distance tested, there were several solutions, depending on the height of the first section considered. For the logs between two disks, the dry weight estimation was calculated from the average dry weight wood ratio of each disk, and for basal and terminal logs the disks immediately above or below the log were considered.

For the *PW* method, it was considered that only one part of the stem was weighed, and for the rest of the tree the volume was calculated from diameter under bark measured every 0.5 m along the stem and by use of the Smalian formula. The length of the weighed and cubed log was made to range between 0.5 m and the total stem height (up to a small-end diameter of 7 cm), considering a variable position of the log along the stem. The fresh weight of the log was transformed to dry weight by considering the moisture content derived from the whole set of disks taken each 0.5 m. Volume to dry weight ratios were then used to estimate the total dry mass of the stem by multiplying by the calculated volumes.

Both methods and sampling intensities were compared with the results obtained by the *CW* method and disk equidistance of 0.5 m, considering the relative difference in the biomass estimation for each tree [3.2].

$$RD = \frac{\left(\hat{W} - W\right)}{W} \cdot 100 \qquad [3.2]$$

where $\hat{W}$ is the predicted bole mass value with each sampling methodology and intensity.

The combinations of inter-disk distances, weighed log lengths and starting point along the bole provided a relative difference value, and these were plotted against subsampling intensity for different diameter classes. The 95% confidence intervals were obtained considering a normal

distribution for different classes of sampling intensity. The default consideration of the bottom disk (*CW* method) or the bottom log (*PW* method) was considered separately for comparison.

3.2.4. Models and fit

Models for predicting biomass of tree components are usually based on the allometric relationship [3.3] between tree biomass and tree variables. This was then used as the basic form of the models to be fitted (Zianis & Mencuccini, 2004).

$$W_i = b_1 \cdot x_1^{b_2} \cdot \ldots \cdot x_n^{b_{n+1}} \qquad [3.3]$$

where W_i is the dry mass biomass of the fraction i and x_n are the independent variables.

The model fitting was carried out in two steps. First, each biomass fraction was fitted individually considering each independent variable and their combinations, by use of the minimum generalized squares in the MODEL procedure of SAS/STAT® (SAS Institute Inc, 2004). As initial parameters in the iteration process, a previous linear fit was carried out for all combinations of variables, with the linearized allometric model (Parresol, 2001), by use of the REG procedure of SAS/STAT®. In selecting the best model for each family of equations, the following statistics were calculated for each equation: bias (*MRES*, [3.4]), root mean square error (*RMSE*, [3.5]) and adjusted determination coefficient ($R^2Adj.$, [3.6]).

$$MRES = \frac{\sum_{i=1}^{N}\left(W_i - \hat{W}_i\right)}{N} \qquad [3.4]$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N}\left(W_i - \hat{W}_i\right)^2}{N-p}} \qquad [3.5]$$

$$R^2 Adj. = \left(\frac{\sum_{i=1}^{N}\left(W_i - \hat{W}_i\right)^2}{\sum_{i=1}^{N}\left(W_i - \overline{W}_i\right)^2}\right) \cdot \left(\frac{N-1}{N-p}\right) \qquad [3.6]$$

where N is the number of data used in the fitting, p is the number of parameters to be estimated, $\overline{W}_i$ is the average value of the dependent variable.

In the second step, each family of equations was fitted simultaneously by the seemingly unrelated regressions method (SUR) to guarantee the additivity of the system (Parresol, 2001).

This method is based on the fit of an apparently non related equation system formed by the regression functions of the biomass fractions considered and the total biomass. The SUR method iteratively forces the sum of the components to equal the equation for total biomass, ensuring that the global solution is the best possible, although the solution for each *k* fraction is not necessarily the best. The independent variables in the system of equations for biomass components has to be the same as in the total biomass equation (Parresol, 2001), and in fact this equation was expressed as the sum of each component equation to ensure the additivity of the system (Álvarez-González *et al.*, 2007). The MODEL procedure of SAS was applied to obtain the SUR estimates, considering the parameters obtained in the individual fitting as initializers.

Lack of homogeneity in error variance, or heteroscedasticity, is commonly observed in biomass equations (Parresol, 1993; 2001). As with the standard errors of the parameter estimates, heteroscedasticity was detected by representing the studentized residuals against the real values, and the White (1980) and Breusch & Pagan (1979) tests were applied. Heteroscedasticity was corrected by weighted fitting (Schaegel, 1982; Clutter *et al.*, 1983; Cunia, 1987; Parresol, 1999; 2001), by use of the inverse of the variance of the residuals (σ_i^2) assigned at each observation as a weighting factor, and use of the potential expression [3.7] (Neter *et al.*, 1989).

$$\sigma_i^{\,2} = x_i^{\,k} \qquad [3.7]$$

The value of the *k* exponent can be calculated by the optimization method proposed by Harvey (1976), which consists of using the model errors fitted without weights ($\hat{e}_i$) as dependent variable in the potential variance error model (Álvarez-González *et al.*, 2007), the linearized form of which is shown in expression [3.8].

$$ln(\hat{e}_i^{\,2}) = a + k \cdot ln(x_i) \qquad [3.8]$$

For each fraction, the value of the *k* exponent was determined for the independent variables or the combination of these that provided the best fit. In those cases in which the statistics did not detect heteroscedasticity, the weighted fit was carried out anyway. The values of the *k* exponents were added to the fitting program in SAS/STAT® (SAS Institute Inc, 2004). After fitting, the models were again subjected to heteroscedasticity tests to verify their correctness.

3.3. Results

3.3.1. Estimation of bole biomass through systematic subsamplig

The relative difference (*RD*) obtained by the *CW* method was plotted against systematic subsampling intensity (disks m^{-1} of stem), for three dimensional classes: DC1 (d<14 cm), DC2

(14<*d*<24 cm) and DC3 (*d*>24 cm) (Fig. 3.3). The figure shows overestimates for all the data, with a clear trend for the relative error to decrease as sampling intensity increased. Small trees are clearly biased towards overestimation (*d*<14 cm), but the tendency for overestimation decreases greatly with increasing tree size. A threshold of 5% relative error would mean a minimum subsampling intensity of 0.95 disks m^{-1} (DC1), 0.8 disks m^{-1} (DC2) or 0.75 disks m^{-1} (DC3). These values were respectively 0.7, 0.4 and 0.3 disks m^{-1}, for a relative error threshold set at 10%. It is important to note that the default consideration of the stem bottom as the position of the first disk would mean a systematic tendency to overestimation.

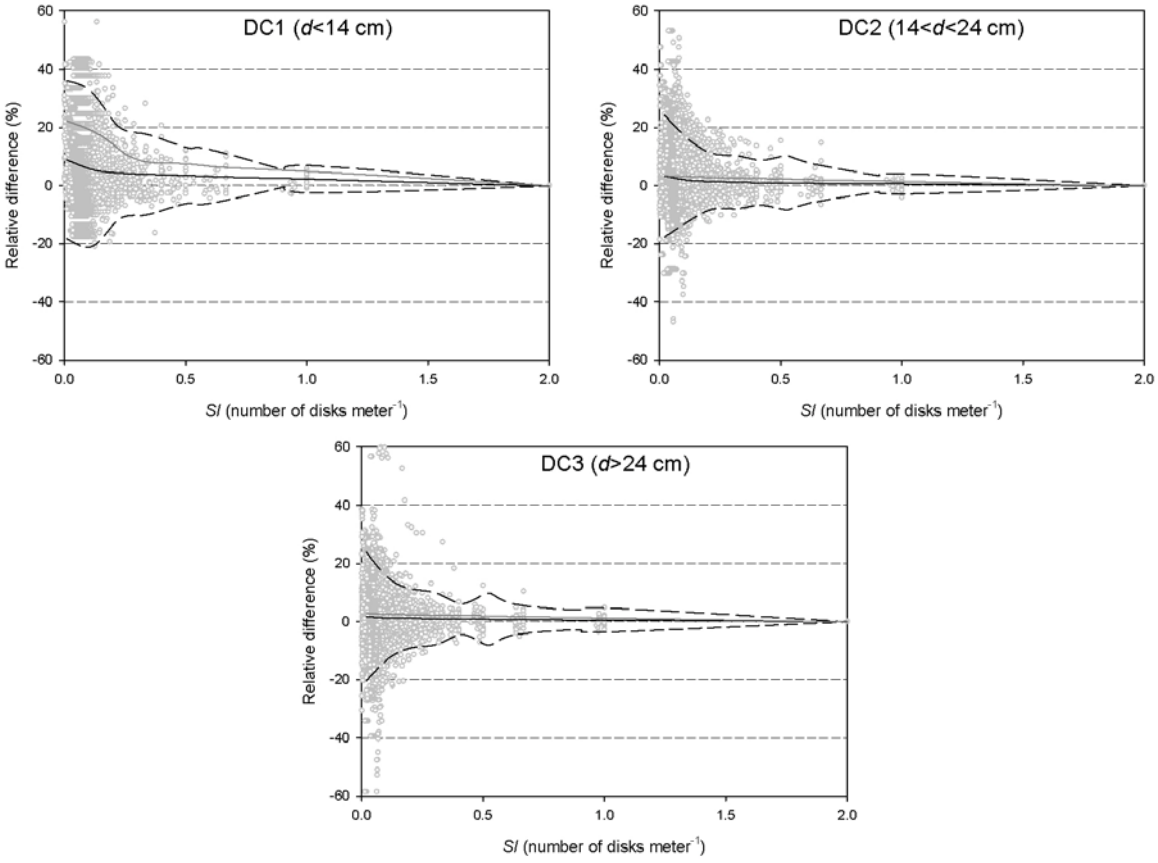

Figure 3.3. Relative difference for three dimensional classes: DC1 (*d*<14cm; *n* = 6 922), DC2 (14<*d*<24cm; *n* = 17075) and DC3 (*d*>24cm; *n* = 18360), plotted against sampling intensity (disks per stem meter) for the *CW* method. Continuous black line: average value for all data; dotted black lines: 95% confidence intervals for all data; continuous grey line: average value for alternatives that include bottom log. *n* is the number of simulated alternatives for each dimensional class.

The *RD* values obtained for the *PW* method were plotted against the subsampling intensity, expressed as the percentage of stem height that was weighed (Fig. 3.4). Only the higher diameter classes were considered in this case, as there is no reason to avoid obtaining the complete fresh weight of small trees. In this case there was again a clear tendency for the weights to be overestimated, which was even clearer for the highest diameter class. This procedure ensured a maximum relative error of 5%, only when 90% of the stem was weighed for both dimensional classes considered. This percentage was 55% when the threshold of relative error was set at 10%.

In this case, consideration of the bottom log as the one that should be weighed systematically led to underestimation.

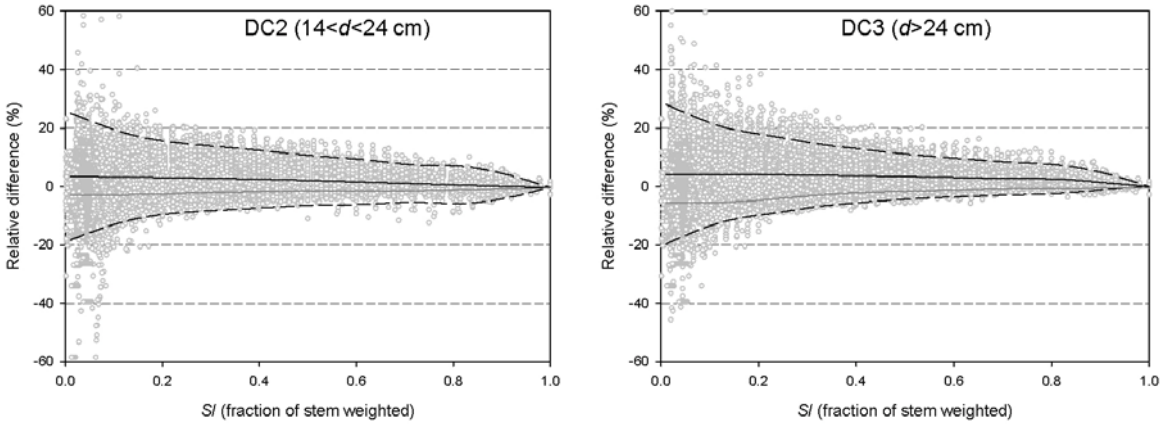

Figure 3.4. Relative difference for two dimensional classes: DC2 (14<*d*<24 cm; *n* = 7712) and DC3 (*d*>24cm; *n* = 12001), plotted against sampling intensity (fraction of stem height weighed) for the *PW* method. Continuous black line: average value for all data; dotted black lines: 95% confidence intervals for all data; continuous grey line: average value for alternatives that include bottom log. *n* is the number of simulated alternatives for each dimensional class.

The differences in the two methods arise from the observed trends in moisture content and basic density along the stem. The moisture content (wet basis) in relation to the relative height along the stem indicates an increasing trend that is more marked at the bottom 20%, where around 40% of bole dry matter occurs (Fig. 3.5). The increasing trend of basic density (ρ, kg m^{-3}) along the stem, which can easily explain the underestimations derived from the default use of the bottom log in the *PW* method, is shown in Fig. 3.6.

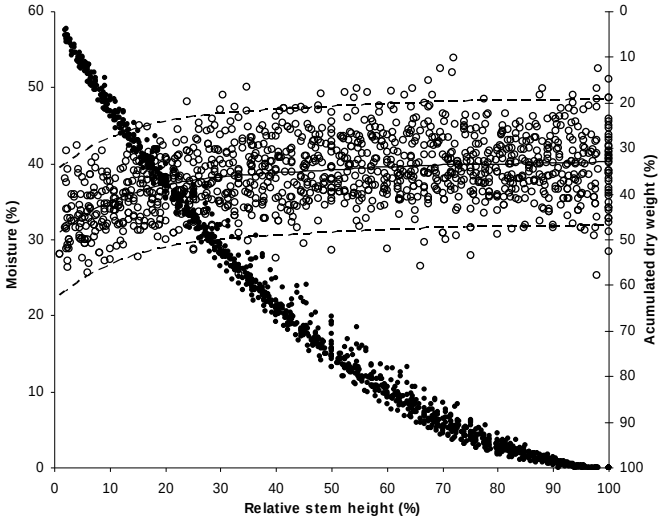

Figure 3.5. Changes in moisture content (%) and accumulated stem dry weight (%) plotted against relative height along the stem (until 7 cm of diameter over bark). Continuous line: average value; dotted lines: 95% confidence intervals.

Such increasing trends, which have been described for different species of eucalypts, with some exceptions, such as for *Eucalyptus regnans*, indicate the possibility of studying the relative

height at which the average basic density can be found. This value was plotted for the sampled trees with non-zero amounts of wood (Fig. 3.7). The relative height tended to decrease with increasing breast height diameter. These results are of great interest for defining the height along the stem that should be sampled to obtain a good estimate of basic density.

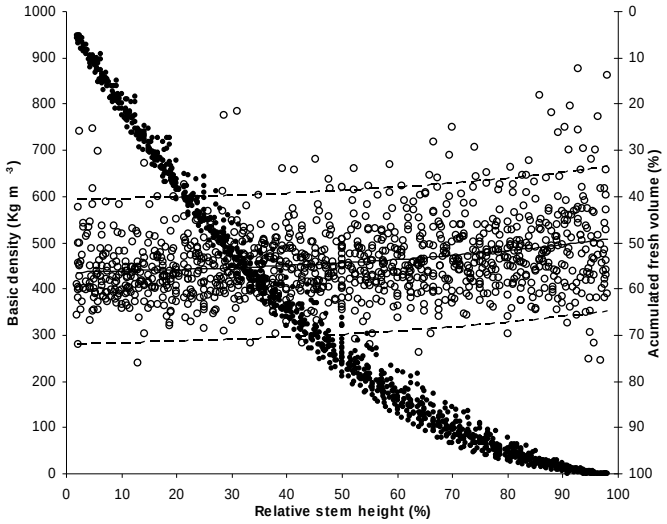

Figure 3.6. Changes in basic density (ρ, kg m^{-3}) and accumulated stem fresh volume (%) plotted against relative stem height (until 7 cm of diameter over bark). Continuous line: average value; dotted lines: 95% confidence intervals.

For the *PW* method, it should be considered that the errors would be cumulative if transformation of fresh weight of the log to dry weight is carried out after obtaining information derived from disks with equidistance greater than 0.5 m. Moreover, the volumes were calculated considering 0.5 m logs, which could provide volume estimates close to those obtained from water displacement of fresh samples (Brown *et al.*, 1995).

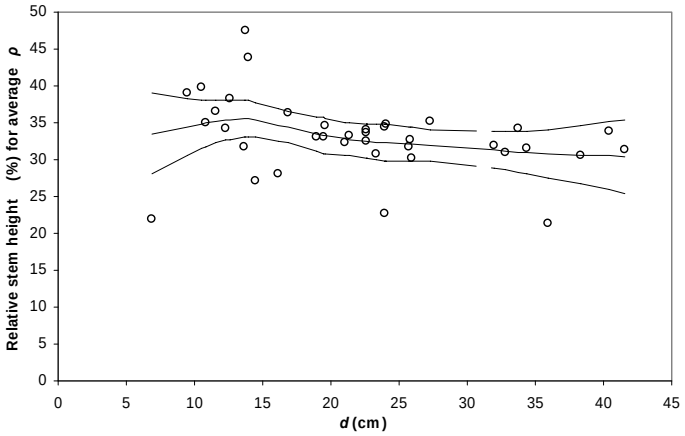

Figure 3.7. Changes in relative stem height (%) where composite average basic density (ρ) is found in relation to breast height diameter of sampled trees. Continuous line: average value; dotted lines: 95% confidence intervals.

3.3.2. Fitting of biomass equations

The first step in developing single tree biomass equations for aboveground biomass components was to fit the allometric model considering one explanatory variable. The best predictive results were obtained with d for all the components, although d_{st} showed good predictive values for W_{db}, W_{Tb} and W_{tb}. There was a clear relationship between all biomass components and d (Fig. 3.8). The combined use of d and h as explanatory variables increased the accuracy of predictions only in the case of the wood component.

The information derived from these fittings was used to consider the best combination of independent variables to add to the allometric model, fitting two sets of equations: stem equations, based exclusively on d and h, and crown equations, in which crown variables were also considered. The models used and the statistics of the simultaneous fitting are shown in Table 3.3. The crown variables with the best predictive ability were crown length and crown diameter. The observed versus predicted values for both set of equations are shown in Figs. 3.9 and 3.10. The weighted adjustment ensured homoscedasticity for all the models fitted.

Predictions of the parameters for the simultaneous fit are shown in Table 3.4. All the exponential parameters were significant, whereas some of the multiplicative parameters were not. This was considered acceptable, as the exponential parameters represent the relative growth of one part of the tree in proportion to another (Broad, 1998), and provide information about the distribution of primary net production among different fractions (António *et al.*, 2007), whereas the multiplicative parameters have not a clear biological meaning.

The explanation of W_{tot} was high for stem variables as a unique estimator, and the combination of d and h provides the best results for this fraction. All crown variables, except h_{cb}, work well individually as predictors for this fraction but the model is less accurate than for stem variables. The inclusion of h_c or v_c as a predictor with h slightly improved the accuracy, but the parameter that affects the crown volume was not significant.

The worst fit was obtained with W_{db}, as already reported by other authors who have studied this fraction in *Eucalyptus* (Saint-André *et al.*, 2005). Fitting of this fraction with crown variables provided a slight increase in accuracy and reduced bias, which is reasonable because almost all dead branches were collected along the stem and h_{cb} is a direct measurement of this length. This suggests that changes in dry weight of this fraction may be more closely related to density than to tree variables.

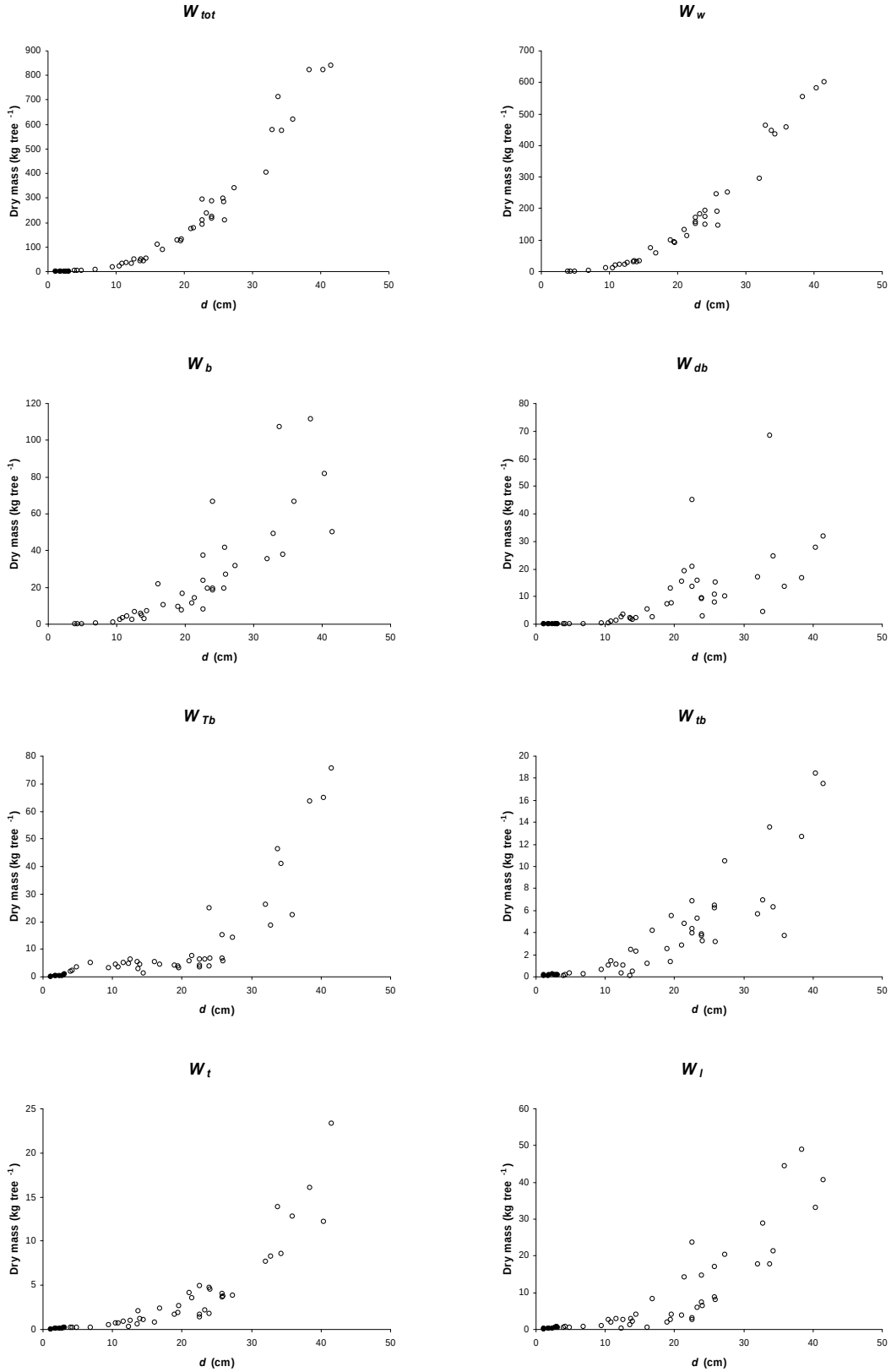

Figure 3.8. Relationship between dry weight of each biomass fraction (kg tree^{-1}) and diameter at breast height (d, cm). Open circles represent trees used in the regression analysis (n = 40), full circles represent additional felled trees for the partitioning study (n = 8).

Table 3.3. Models selected for simultaneous fitting of each equation system.

	Stem equation system				*Crown equation system*			
Fraction	Model	*RMSE*	*MRES*	$R^2 Adj$	Model	*RMSE*	*MRES*	$R^2 Adj$
W_{tot}	$W_{tot} = \sum W_i$	37.9	2.86	0.98	$W_{tot} = \sum W_i$	39.9	1.36	0.97
W_{db}	$b_{1.1} \cdot d^{b_{1.2}}$	10.6	0.32	0.40	$b_{2.1} \cdot d^{b_{2.2}} \cdot h_{cb}^{\ b_{2.3}}$	10.4	-0.04	0.42
W_b	$b_{1.3} \cdot d^{b_{1.4}}$	15.3	0.72	0.71	$b_{2.4} \cdot d^{b_{2.5}}$	15.3	0.30	0.71
W_w	$b_{1.5} \cdot d^{b_{1.6}} \cdot h^{b_{1.7}}$	17.9	0.32	0.99	$b_{2.6} \cdot d^{b_{2.7}} \cdot h^{b_{2.8}}$	18.2	-0.16	0.99
W_{Tb}	$b_{1.8} \cdot d^{b_{1.9}}$	6.0	1.05	0.90	$b_{2.9} \cdot d^{b_{2.10}} \cdot d_c^{\ b_{2.11}}$	5.4	0.62	0.92
W_{tb}	$b_{1.10} \cdot d^{b_{1.11}}$	2.2	0.13	0.76	$b_{2.12} \cdot d^{b_{2.13}}$	2.4	0.24	0.71
W_t	$b_{1.12} \cdot d^{b_{1.13}}$	2.1	0.07	0.83	$b_{2.14} \cdot d^{b_{2.15}} \cdot d_c^{\ b_{2.16}}$	1.7	0.26	0.89
W_l	$b_{1.14} \cdot d^{b_{1.15}}$	5.6	0.25	0.81	$b_{2.17} \cdot d^{b_{2.18}} \cdot h_c^{\ b_{2.19}}$	4.6	0.15	0.87

Definitions of the different fractions are given in section 3.2.2. W_{tot} refers to total aboveground biomass.

W_b was more closely related to stem than to crown variables, and no additional improvements in estimations were obtained by addition of other variables. No crown variables were necessary for a good fit of W_w.

The inclusion of d_c as a crown variable provided a 10.8% increase in accuracy (measured as *RMSE*, Eq. [3.5]) and reduced bias (Eq. [3.4]) by 41.4% for the W_{Tb} explanation (data not shown). The overall results from use of only stem variables were good, and the adjusted coefficient of determination reached as high as 89.7%. In the case of thin branches, the simultaneous fitting with crown variables provided poorer fits.

The explanation of W_{tb} was high with d as the only estimator, the inclusion of h did not improve the accuracy and the estimation of its respective parameter was not significant. All crown variables were individually poorer estimators than d, but inclusion of d_c or v_c provided a slight improvement in accuracy, although respective parameters were not significant.

The use of crown diameter in the prediction of W_t increased the accuracy by 19.1%, although the bias was higher for the crown equation system. The crown height also improved the accuracy of estimation of W_l by 17.3%. Crown variables by themselves explained more than 59% of the variability in W_l. Estimation of these two components and W_{Tb} was greatly improved by use of crown variables.

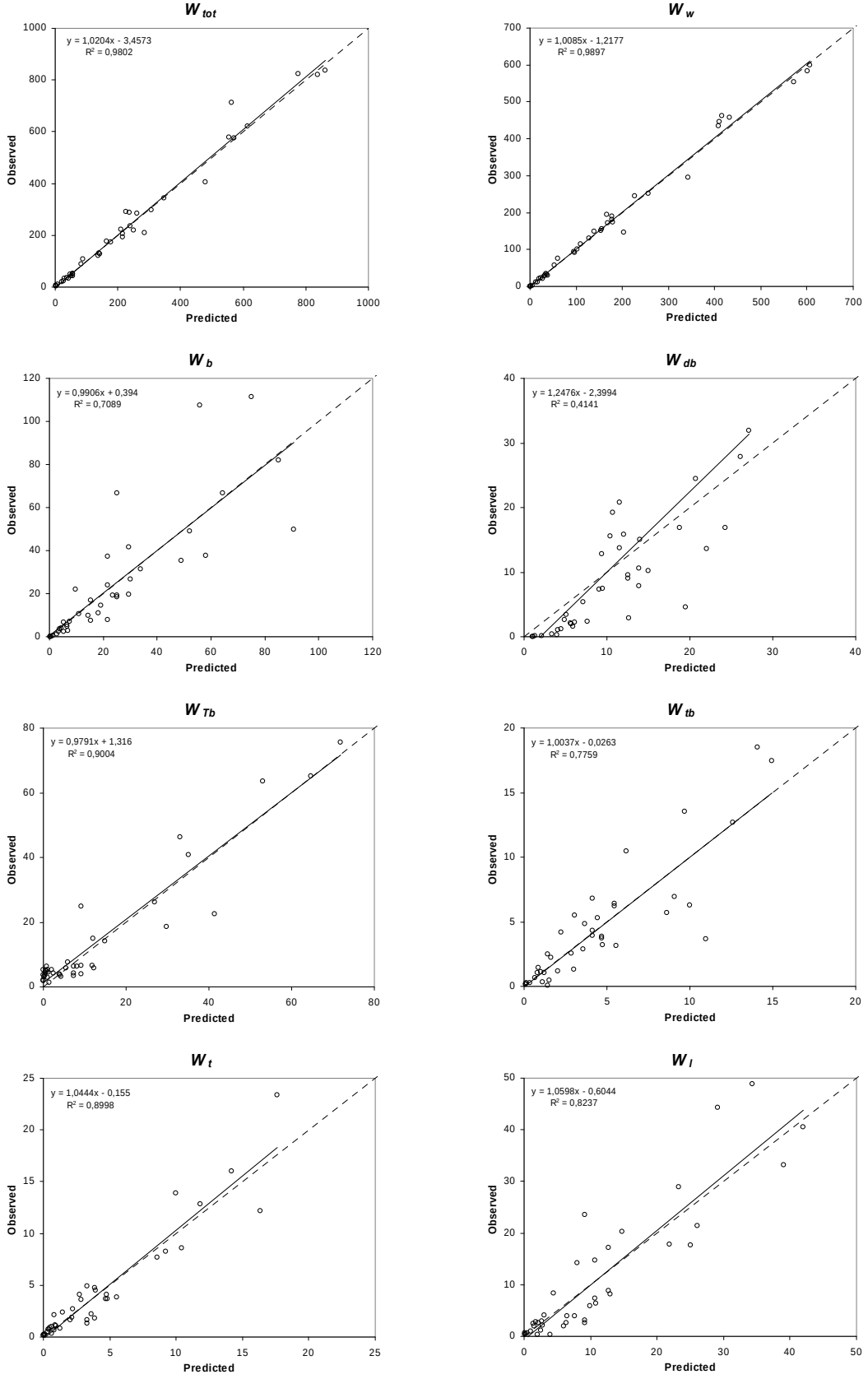

Figure 3.9. Relationship between observed-predicted dry weight values for each biomass component (kg tree^{-1}) in the Stem system of equations.

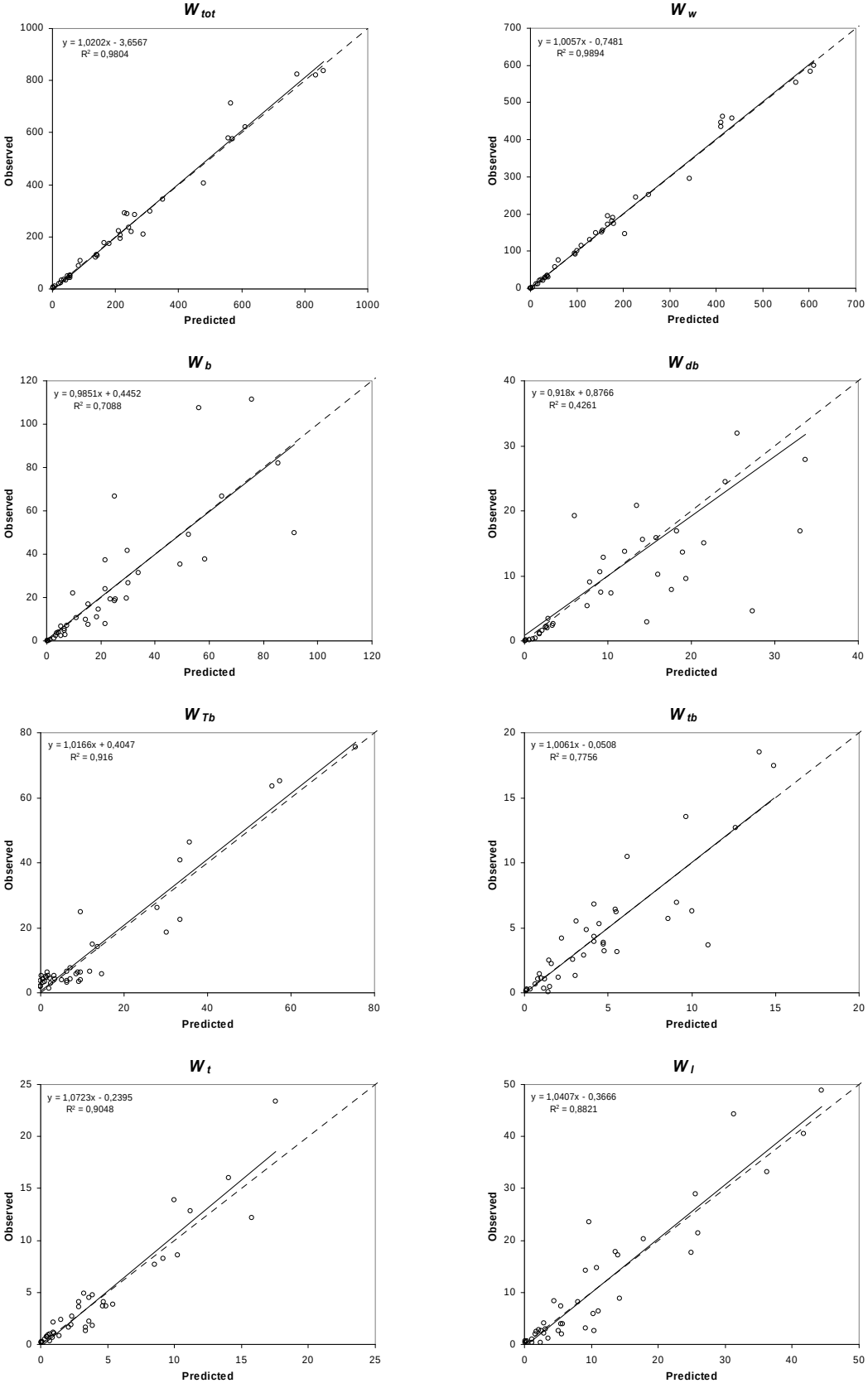

Figure 3.10. Relationship between observed-predicted dry weight values for each biomass component (kg tree^{-1}) in the Crown system of equations.

Table 3.4. Parameters for simultaneous fitting of equations.

Parameter	Estimate	Appr.*SE*	Pr > |*t*|	Parameter	Estimate	Appr.*SE*	Pr > |*t*|
$b_{1.1}$	0.145	0.05	0.0063	$b_{2.1}$	0.0079	0.0077	0.3137
$b_{1.2}$	1.403	0.12	<.0001	$b_{2.2}$	1.279	0.313	0.0003
$b_{1.3}$	0.013	0.0083	0.1177	$b_{2.3}$	1.254	0.411	0.0044
$b_{1.4}$	2.361	0.1892	<.0001	$b_{2.4}$	0.0318	0.016	0.0545
$b_{1.5}$	0.0094	0.0024	0.0004	$b_{2.5}$	2.1079	0.156	<.0001
$b_{1.6}$	2.0329	0.082	<.0001	$b_{2.6}$	0.0149	0.0034	0.0001
$b_{1.7}$	1.0562	0.1335	<.0001	$b_{2.7}$	2.0515	0.081	<.0001
$b_{1.8}$	0.000059	0.000064	0.3586	$b_{2.8}$	0.8946	0.128	<.0001
$b_{1.9}$	3.7599	0.2983	<.0001	$b_{2.9}$	0.00082	0.0010	0.4124
$b_{1.10}$	0.0128	0.005	0.0153	$b_{2.10}$	2.6444	0.4403	<.0001
$b_{1.11}$	1.8579	0.131	<.0001	$b_{2.11}$	0.7627	0.265	0.0069
$b_{1.12}$	0.00092	0.00049	0.07	$b_{2.12}$	0.030047	0.0098	0.0042
$b_{1.13}$	2.6322	0.159	<.0001	$b_{2.13}$	1.590388	0.1168	<.0001
$b_{1.14}$	0.0053	0.0034	0.1281	$b_{2.14}$	0.006228	0.0028	0.0329
$b_{1.15}$	2.3931	0.197	<.0001	$b_{2.15}$	1.949093	0.1932	<.0001
				$b_{2.16}$	0.218899	0.01909	0.0259
				$b_{2.17}$	0.016847	0.0102	0.109
				$b_{2.18}$	1.515742	0.2651	<.0001
				$b_{2.19}$	0.774688	0.1934	0.0003

3.3.3. Proportions of each biomass component

The statistics for the dry weight biomass fractions considered in the present study are shown in Table 3.2. Wood is particularly important in the total biomass, representing about 70% of total dry weight for the average tree size, which emphasizes the importance of its accurate estimation. The next fractions in importance are bark (10%) and thick branches, dry branches, leaves, thin branches and twigs. The relative proportions of each component, plotted against diameter, including a set of 8 small trees which were not used for fitting, are shown in Fig. 3.11. The proportion of some of these components in trees of different diameter class have been used as parameters in physiological growth models, and accurate estimation by use of the models proposed in this paper is desirable.

The proportion of each component related to total aboveground biomass is becoming critical in a scenario of increasing harvesting of biomass components that were previously left in place in forest soils. The proportion of wood, commonly referred to as the harvest index, increased with diameter (Fig. 3.11), although the trend was not continuous because of the need to consider a threshold diameter. Consequently, the component of thick branches may account for a large share of total aboveground biomass for trees with a diameter still too small to have a significant wood fraction. The bark fraction was defined as the bark fraction in the stem, and the bark of sections less than 7 cm in diameter was included in thick or thin branches, which explains the low

percentages shown for diameters less than 12 cm. As a result, the equations presented here would provide reasonable estimates of biomass component percentages for diameters larger than 12 cm. For smaller diameters, exclusive use of the equation predicting total biomass is recommended.

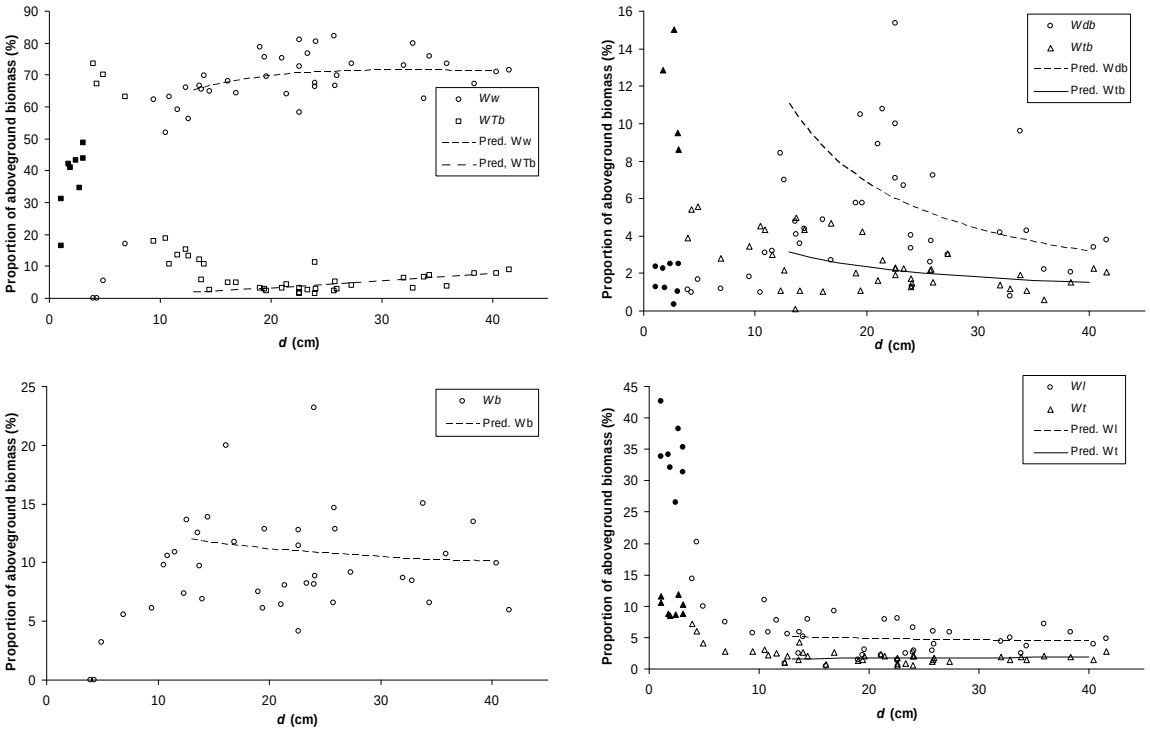

Figure 3.11. Proportion of each biomass fraction over total aboveground biomass. Open figures: trees used in developing biomass equations; filled figures: additional small trees; lines: prediction of biomass equations.

3.4. Discussion

3.4.1. Stem biomass estimation

The results of this study show that the error may be important, and will depend on the intensity of subsampling, when ratio-type estimators are used to estimate dry mass. The error also depends on the method used (complete fresh weight or partial fresh weight) and on the average tree size. Other authors have observed that ratio-type estimators provide biased estimates (Cunia, 1979; Valentine *et al.*, 1984; Briggs *et al.*, 1987). These overestimates are as large as the decreases in both subsampling intensity and average tree size. Overestimation is clearly a more serious error than underestimation (Satoo & Madgwick, 1982) because it does not err on the side of safety i.e. for carbon accounting procedures.

Wood moisture content and basic density change along the stem (Satoo & Madgwick, 1982) (Figs. 3.5 and 3.6), and affect the estimation of dry biomass by the *CW* and *PW* methods respectively. Minimum moisture content and basic density occur in the basal part of the stem, which is obviously where most of the accumulated weight and volume occur. This effect must therefore be taken into account with a sufficient and well distributed number of subsamples along the stem. One way of addressing this problem, when taper functions are available, is the density integral approach (Parresol & Thomas, 1989). The weighed average is an alternative method that gives more importance to those observations in the lower part of the stem, and therefore more closely related to volume.

Chave *et al.* (2001) reported that the biomass values of the smallest trees strongly affect the values of the model parameters in the allometric relation. This effect is even stronger when a weighted adjustment methodology is used, because the smallest trees, which are less variable, are more important than the largest trees because of heteroscedasticity correction. It is therefore advisable to obtain the complete dry weight of the stem of small trees.

The degree of accuracy required depends on the objective of the estimation, although equilibrium between sampling intensity and the level of precision must be ensured (Brown *et al.*, 1995). If ratio-type estimators are chosen for stem dry biomass estimation, a relatively intensive subsampling scheme should be implemented, as others authors indicated for both ratio-type and density-integral methods (Parresol, 1999). Comparing the methods considered here, the *CW* method produced better results for the largest dimensional class than the *PW* method (Figs. 3.3 and 3.4). This is because, for a given length of cubed and weighed part, the proportion over total stem (as an indicator of sampling intensity) differs depending on tree size, and therefore becomes less important as tree size increases. This must be taken into account because the *PW* method is usually used for large trees in which complete weighing is time-consuming.

The results clearly show the trends in relative errors derived from a default consideration of the bottom disk or the bottom log as the first section to measure. It is advisable, if systematic sampling is to be used, to establish the subsampling intensity before randomizing the position along the stem of the first disk or log to be measured. In the case of the *PW* method it is not recommended to take only one sample log per tree, although this was the approach used in this study. The subsampling intensity should be split along the stem, and a good representation of the bole area where average basic density is likely to be found is advisable. Most published papers do not provide information about the proportion of weighed and cubed logs or their distribution along the stem, although the most reasonable distribution would be systematic or random, with the subsampling intensity chosen on the basis of statistical criteria.

3.4.2. Biomass equations from stem and crown variables

Although it is known that *d*, *h* and *W* are closely related (Satoo & Madgwick, 1982), *h* is not always included in biomass equations together with *d* because both are correlated and inclusion of

h adds only negligible accuracy (Jokela *et al.*, 1986; Ter-Mikaelian & Korzukhin, 1997; Johansson, 1999; Verwijst & Telenius, 1999; Snowdon *et al.*, 2000; Brown, 2002; Porté *et al.*, 2002; Jenkins *et al.*, 2003). In this study, inclusion of *h* together with *d* only resulted in improved accuracy in the case of wood, although other authors have reported significant improvement for several fractions (Loomis *et al.*, 1966; Pearson *et al.*, 1984; Bartelink, 1996; Reed & Tomé, 1998; Monserud & Marshall, 1999). In their study on *Eucalyptus globulus*, António *et al.* (2007) observed improvements in the sum of residual squares of 72%, 8%, 12% and 10% for wood, bark, leaves and branches respectively, after inclusion of *h* together with *d*. It is possible that in the present study the tree sample was not representative of the entire variability in height for a given diameter.

Some studies included *h* and *d* in biomass models, together with density, age and site index (Ter-Mikaelian & Parker, 2000; António *et al.*, 2007), and these models are therefore suitable for comparing different sites (Ketterings *et al.*, 2001). Other studies included age as an independent variable in biomass equations (Porté *et al.*, 2002; Saint-André *et al.*, 2005), thus producing dynamic models with which biomass increments can be estimated by derivative analysis. Although individually d_{st} worked well as a predictor, it is seldom measured in forest inventories. On the other hand, it is sometimes useful to estimate dry biomass when trees are already cut down and only stump dimensions are available.

It has been observed that some crown variables work well as predictors of crown fractions (Clark, 1982; Satoo & Madgwick, 1982; Carvalho & Parresol, 2003). In the present study, inclusion of crown variables improved the *RMSE* by 1.8%, 10.8%, 19.1% and 17.3% for respectively dead branches, thick branches, twigs and leaves, in the individual fit. These improvements are smaller than those obtained by António *et al*. (2007) for *Eucalyptus globulus* in Portugal, probably because of the lower genetic variability in the plantations considered in our study. The best improvement was for leaves, which implies better estimations of a fraction that is very difficult to predict and is very important as regards nutrition and ecology. Overall, the results indicate a low accuracy of estimation of the bark fraction in the present study, in comparison with reports for other species of *Eucalyptus*. Wood, bark and thin branches depend on the same variables in both systems of equations, and the results obtained by simultaneous fitting were generally only slightly less accurate. For leaves, the reduction in $R^2Adj.$ derived from simultaneous fitting was 6.3%.

The ability of the fitted biomass equations to evaluate the proportion of each aboveground biomass component for a range of diameters has seldom been studied. The proportions are often considered as parameters for ecophysiological models, particularly for small diameters (Sands & Landsberg, 2002). The present results show that, if a threshold diameter is considered for defining a wood component, minimum breast height diameter must be considered to define the range of use of the biomass components equations, if sound estimation of these percentages is sought.

3.5. Conclusions

Two systems of equations were fitted for aboveground biomass components of *Eucalyptus nitens*. The inclusion of crown variables as predictive variables provided poorer results for total biomass, wood and thin branches, but improved the accuracy of estimation for twigs, leaves, thick branches and dead branches.

Stem subsampling affects estimation of the wood fraction. If a systematic subsample of disks or logs is taken, the variation in moisture content or basic density along the stem should be considered. Less intensive sampling usually leads to overestimation of biomass with both methods (complete fresh weighing or partial fresh weighing). The minimum subsampling intensity for an assumed ±5% *RD* in wood dry biomass estimation depends on the tree diameter class, with a range of 0.75 to 0.95 disks m^{-1} in the *CW* method. Use of the *PW* method would require very intense subsampling to reduce the relative error, independently of tree size. The average basic density usually occurs at a relative height of 30-35% along the stem. It is not always advisable to choose the first section of study at the bottom of the stem.

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Chapter IV

A management tool for estimating bioenergy production and carbon sequestration in Eucalyptus globulus *and* Eucalyptus nitens *grown as short rotation woody crops in north-west Spain*

4. A management tool for estimating bioenergy production and carbon sequestration in *Eucalyptus globulus* and *Eucalyptus nitens* grown as short rotation woody crops in north-west Spain

Abstract

This study proposes stand level models for estimating biomass yield, total energy and carbon sequestration in *Eucalyptus globulus* and *Eucalyptus nitens* plantations, on the basis of measurements made in 131 plots established at the usual range of initial forest densities for southwestern Europe. The timber volume, total aboveground biomass, logging residue biomass, crown biomass, carbon in aboveground biomass and soil organic layer, energy in aboveground biomass, energy in logging residue biomass and usable cellulose yield were represented in the form of isolines (taking mortality into account) and plotted against dominant height. These variables were calculated and compared with previously published data on two silvicultural options for short rotation forestry, one destined for bioenergy production and the other consisting of the standard silviculture regime applied to both species in southern Europe, considering the average site index for each species. Yield levels were higher in *Eucalyptus nitens* than in *Eucalyptus globulus* for all variables because of faster diameter increment at similar densities. The total yield in terms of biomass was 13.9-14.6 Mg ha^{-1} yr^{-1} for *Eucalyptus globulus* and 20.4-21.5 Mg ha^{-1} yr^{-1} for *Eucalyptus nitens*. Energy in aboveground biomass ranged between 233 and 245 GJ ha^{-1} yr^{-1} for *Eucalyptus globulus* and 345 and 364 GJ ha^{-1} yr^{-1} for *Eucalyptus nitens*, carbon accumulation rate in aboveground biomass and soil organic layer was 6.9-7.2 Mg ha^{-1} yr^{-1} for *Eucalyptus globulus* and 12.7-13.5 Mg ha^{-1} yr^{-1} for *Eucalyptus nitens*, and usable cellulose was 5.7-5.9 Mg ha^{-1} yr^{-1} for *Eucalyptus globulus* and 9.0-10.1 Mg ha^{-1} yr^{-1} for *Eucalyptus nitens*. It was found that 50% increments in the initial density result in only marginal increments in biomass and usable cellulose yields.

Keywords: short rotation forestry, eucalypts, woody crops, density management diagrams, bioenergy production, carbon sequestration.

4.1. Introduction

Short rotation woody crops (SRWCs) (grown in short rotation forestry: SRF) are an important potential source of cellulosic biomass, which can be used as solid fuel in the form of wood chips,

pellets or charcoal, transformed into ethanol via a cellulosic platform and/or used in pyrolysis to generate syngas and other products (Johnson *et al.*, 2007). The establishment of single stem stands and subsequent replanting or coppicing is the most common management regime in SRF, and decisions about replanting and coppicing should consider: (i) the yields from both options (ii) establishment costs, and (iii) desired dimensions of the final product (Rockwood *et al.*, 2006).

The genus *Eucalyptus* has been used in forestation in Europe since the early 19th century because of its high productivity and plasticity. The total area currently occupied by *Eucalyptus* plantations in southern Europe is approximately 14000 km^2, with *Eucalyptus globulus* being the most common species but with an increasing proportion of *Eucalyptus nitens*, which is grown successfully as a frost-tolerant species. Both species belong to the subgenus *Symphyomyrtus*, known to produce larger average tree sizes and to be more productive than species of the subgenus *Monocalyptus* (Davidson & Reid, 1980; Turnbull *et al.*, 1993; Sims *et al.*, 1999a). The management objective of these plantations in southern Europe is currently the production of wood pulp or fibreboard, although logging residues and the bark derived from the harvesting operations are increasingly used as biofuel to produce thermal energy and electricity.

The ideal characteristics of an energy crop are: (i) high yield, (ii) low energy input for production, (iii) low cost, (iv) minimal contents of contaminants and (v) low nutrient requirements (McKendry, 2002). In this sense, *Eucalyptus* species adapt well to energy production, because of the high yield and low water and nutrient requirements than for poplars and willows (Johnson *et al.*, 2007); *Eucalyptus* species accounted for 38% of total SRF plantations throughout the world in 2003 (FAO, 2003). Before establishment of a plantation it is important to consider the desired combination of stem density and rotation. For species that must be established from seedlings, the application of narrow inter- and intra- row spacing would lead to very high costs and low ratios of wood/other biomass components, wood/bark or percentage of cellulose. Moreover, the average size of tree decreases as *N* increases (Bullard *et al.*, 2002) and larger logs are more dense. Nutrient depletion is also less likely for longer rotations, which also provide product flexibility (Ericsson, 1994; Guo *et al.*, 2002). Eucalypts in southern Europe established at initial densities of 1000 to 2400 stems per ha can therefore supply the bioenergy industry as the main plantation objective or through the use of logging residues for energetic purposes.

Forest plantations develop from a collection of individual, freely growing trees, through the onset of competition, to full site occupancy and self-thinning. Stand development is commonly displayed as a trajectory of increasing mean tree size with decreasing stand density (Long *et al.*, 2004). Dynamic stand density management diagrams (SDMDs) illustrate the relationships among yield, density and density-dependent mortality at all stages of stand development. Their use has been proven to be an effective method for the design, display and evaluation of alternative density management regimes in the field of even-aged forestry (Newton, 1997). The adaptation of such management tools to the field of short rotation forestry for bioenergy production may assist in the assessment of energy yield potential, optimum stand management in terms of density and rotation in comparison with other potentially useful woody species.

Yield level indicators are considered the most important factors regarding the suitability of a tree species growing in SRF (Sims *et al.*, 2001). Statistic growth models based on a wide range of empirical data are increasingly used in research on SRF yields, rather than growth trials (Dickmann, 2006), because results from small plots may not be representative (Mitchell *et al.*, 1999; Sims *et al.*, 1999b; Dickmann, 2006). SDMDs are based on relatively large plots established on commercial plantations and therefore provide more realistic yield estimations.

The objectives of the present study were to provide a management tool for estimating biomass and bioenergy production and carbon sequestration in *Eucalyptus globulus* and *Eucalyptus nitens* planted at the observed range of initial stockings in southern Europe, and to propose and compare two standard crop management regimes for both species.

4.2. Materials and methods

4.2.1. Source of data

For development of SDMD, a network of 131 unthinned plots was established in northwest Spain, 55 plots of *Eucalyptus globulus* and 76 of *Eucalyptus nitens*. The plots are located in a temperate Atlantic zone, with an average annual precipitation of 1100-1500 mm for both species and an average annual temperature of 13.9ºC and 13.2ºC, respectively. Average winter temperature is 10ºC for *Eucalyptus globulus* and 8.2ºC for *Eucalyptus nitens*. The coordinates of the plots were 43º41'80''- 42º52'00'' N and 8º24'11''- 7º03'56'' W (Fig. 4.1).

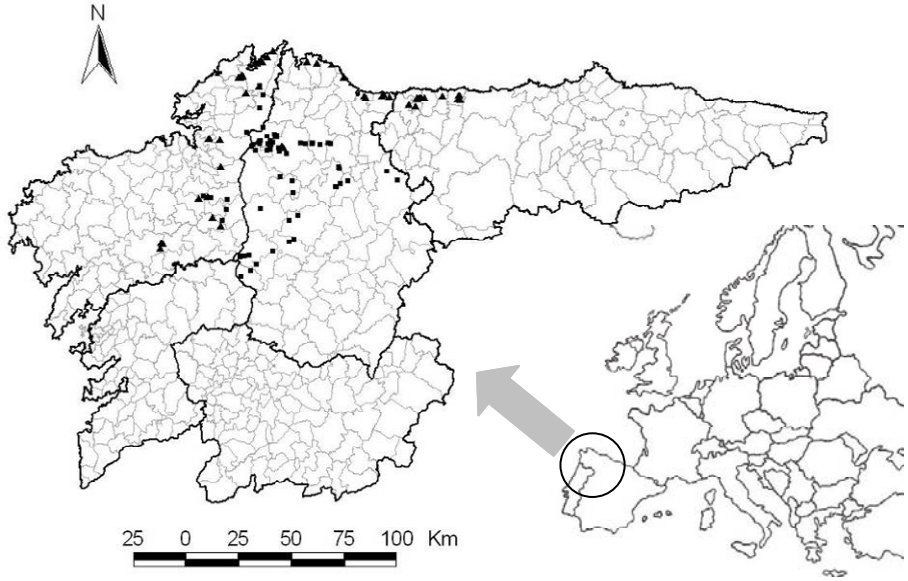

Figure 4.1. Location of the sample plots. *Eucalyptus globulus* (triangles) and *Eucalyptus nitens* (squares).

The plots were measured once (in winter) between 2006 and 2010, thus covering a chronosequence in the range 0 to 25 years. Two types of plots were considered: circular plots of radius 10 m and square plots of 20x20 m, a size well above the threshold of 100-150 m^2 established for accurate biomass estimation (Specht & West, 2003). The diameter at breast height (*d*, cm) and total height (*h*, m) were measured in all trees in each plot. Diameters were measured twice (measurements at right angles to each other) to the nearest 0.1 cm, with calipers, and the arithmetic mean value was calculated. Height was measured with Vertex IV to the nearest 10 cm. The stand variables calculated directly from these measurements were: quadratic mean diameter (d_g, cm), average stand diameter ($\overline{d}$, cm), average stand height ($\overline{h}$, m) and tree density (*N*, stems ha^{-1}).

Dominant height (H_0, m) was determined per plot by use of a local height-diameter relationship and considering the 100 thickest trees per hectare (Assmann, 1970). Site index (*SI*, m) was calculated from local models (Fernández López, 1982; Pérez-Cruzado, 2009), known to be valid for single stem rotation in northern Spain. As the reference ages in these models are not the same (10 and 6 years for *Eucalyptus globulus* and *Eucalyptus nitens* respectively), the *SI* values cannot be directly compared. The volume of each tree was determined from taper functions (Sánchez *et al.*, 2004; Pérez-Cruzado, 2009). The values were added to determine the stand volume (V, m^3 ha^{-1}).

4.2.2. Biomass and energy calculations

The total aerial biomass, logging residue biomass (branches and stem bark) and crown biomass (leaves and branches), were determined from single tree biomass equations developed in Chapter III for *E. nitens*, and fitted with data for *E. globulus* (Brañas *et al.*, 2000), which are shown in Table 4.1. Both systems of equations were fitted simultaneously for samples of 36 *E.globulus* trees and 40 *E. nitens* trees respectively, by the seemingly unrelated regression method (*SUR*) and the MODEL procedure of SAS/ETS® (SAS Institute Inc, 2004).

The following biomass stand variables were calculated: total aerial biomass (*W*, Mg ha^{-1}), logging residue biomass (W_w, Mg ha^{-1}), considered as all the compartments except for stem and leaves and crown biomass (W_c, Mg ha^{-1}), in this case for all the compartments except stem, bark and dead branches under the crown. These values were calculated by summing the individual tree values corresponding to the compartments of interest.

The energy in aboveground biomass (*E,* TJ ha^{-1}) and energy in logging residues (E_w, TJ ha^{-1}) were determined by the low heating value (*LHV*, kJ kg^{-1}), calculated from expression [4.1] from the high heating value (*HHV*, kJ kg^{-1}) and the proportion of hydrogen in the dry matter (H_d, %) reported by Pérez *et al.* (2006) and Pérez *et al.* (2008) for both species. The energy values were first determined at tree level as for the other variables.

Table 4.1. Biomass equations for *Eucalyptus globulus* and *Eucalyptus nitens* in north-west Spain.

Species	Fraction	R^2*Adjust.*	*RMSE* (kg)	*CC* (%)
Eucalyptus globulus	$w_w + w_{lb} = 0.01308 \cdot d^{1.870} \cdot h^{1.172}$	0.989	13.4	45.2
	$w_b = 0.01010 \cdot d^{2.484}$	0.932	3.82	42.5
	$w_{mb} + w_{sb} = 0.003685 \cdot d^{2.654}$	0.841	5.16	45.3
	$w_t = 0.01258 \cdot d^{1.705}$	0.586	0.971	46.4
	$w_l = 0.02949 \cdot d^{1.917}$	0.706	4.38	52.0
Eucalyptus nitens	$w_w + w_{lb} = 0.009400 \cdot d^{2.033} \cdot h^{1.056}$	0.990	17.9	50.1
	$w_b = 0.01342 \cdot d^{2.361}$	0.710	15.3	46.9
	$w_{mb} = 0.00005900 \cdot d^{3.760}$	0.897	6.01	49,8
	$w_{sb} = 0.01280 \cdot d^{1.858}$	0.756	2.24	49.8
	$w_t = 0.0009220 \cdot d^{2.632}$	0.831	2.09	51.5
	$w_l = 0.005300 \cdot d^{2.393}$	0.813	5.59	57.2
	$w_{db} = 0.1451 \cdot d^{1.403}$	0.397	10.6	50.4

where w_w is the wood biomass (up to 7 cm diameter over bark), w_b is the bark biomass in the stem, w_{lb} is the biomass of large branches (up to 7 cm diameter over bark), w_{mb} is the biomass of medium sized branches (diameters over bark between 7 and 2 cm), w_{sb} is the biomass of small branches (diameters over bark between 2 and 0.5 cm), w_t is the twig biomass (diameters over bark smaller than 0.5 cm), w_l is the leaf biomass, and w_{db} is the biomass of dead branches in the bottom stem (a large fraction of the weight in *Eucalyptus nitens*). The carbon concentrations for *Eucalyptus globulus* were obtained from Brañas *et al*. (2000).

$$LHV = HHV - \left(\frac{2442.9 H_d}{100} \right) \quad [4.1]$$

The usable cellulose (*UC,* Mg ha^{-1}) was estimated from the volume under bark data for each plot, and the average specific consumption of wood for pulp production, which is usually evaluated as inside bark wood volume (m^3) required to produce a dry weight of pulp, considering an average value of 3.2 m^3 Mg^{-1} for *E. globulus*, and 3.8 m^3 Mg^{-1} for *E. nitens* (Cotterill & Macrae, 1997).

4.2.3. Carbon estimation

The concentrations of carbon in each biomass component were determined for the sample of trees used to derive biomass equations, by ignition in a LECO CNS-ICP 2000 element analyzer. Carbon in aboveground biomass was calculated from biomass equations and the carbon concentration at tree level (Table 4.1) was calculated by adding the values at plot level and expressing the values per hectare.

The carbon accumulated in soil organic layer was evaluated in only 40 plots per species by collecting 5 random samples per plot in square containers (30x30 cm) and determining the oven

dry weight per unit surface area. The concentrations of carbon in litter were determined after ignition in a LECO CNS-ICP 2000 element analyzer, and average values of 46.94% and 47.68% were obtained for *E. globulus* and *E. nitens* respectively (Chapter II).

Total aboveground carbon (*C*, Mg ha^{-1}) was then calculated for each plot as the sum of the total carbon in aboveground biomass and the carbon in the soil organic layer. Summary statistics, including the mean, maximum, minimum and standard deviation for each of the main stand variables calculated are shown in Table 4.2.

Table 4.2. Statistics for the main variables measured in the sample plots.

Variable	*Eucalyptus globulus*				*Eucalyptus nitens*			
	Mean	Min.	Max.	S.D.	Mean	Min.	Max.	S.D.
d_g (cm)	16.32	1.291	36.44	8.01	19.60	6.452	35.23	5.39
G (m^2 ha^{-1})	27.80	0.092	81.98	20.00	35.33	2.185	81.98	16.92
$\bar{d}$ (cm)	15.29	1.200	35.64	7.67	18.66	6.248	34.06	5.20
$\bar{h}$ (m)	17.35	1,.268	32.07	7.92	19,.61	5.857	32.07	5.23
N (stems ha^{-1})	1182	446	1825	318	1152	446	2196	387
H_0 (m)	22.86	1.10	40.50	10.44	24.54	7.40	40.41	7.08
SI (m)	23.33	8.99	36.32	6.47	16.30	8.80	26.20	4.90
V (m^3 ha^{-1})	291.18	0.064	1139.90	252.50	365.82	8.133	1129.46	223.86
W (Mg ha^{-1})	171.92	0.084	709.12	153.15	187.09	4.763	653.50	119.40
W_w (Mg ha^{-1})	28.88	0.033	110.51	24.77	43.87	2.456	132.30	24.08
W_c (Mg ha^{-1})	20.34	0.052	70.32	16.13	22.93	0.727	85.57	14.82
C (Mg ha^{-1})	101.12	0.040	325.84	74.17	106.92	26.608	358.51	65,.11
E (TJ ha^{-1})	2.88	0.001	11.93	2.58	3.20	0.081	11.17	2.04
E_w (TJ ha^{-1})	0,.45	0.001	1.74	0.39	0.71	0.040	2.14	0.39
UC (Mg ha^{-1})	71.62	0.009	305.55	65.46	82.80	1.678	262.29	51.72

4.2.4. Development and construction of Stand Density Management Diagrams

The model developed in this paper for constructing SDMD includes a system of 9 equations, a density index and the mortality model as basic components. In the system of equations [4.2-4.10], N and H_0 are exogenous variables (defined independently of the system), V, W_w, W_c, C, E, E_w and UC are the endogenous variables (variables that the model is intended to explain or predict), and d_g is the endogenous instrumental variable (Borders, 1989). The first equation [4.2] relates quadratic mean diameter to the number of stems per hectare and the dominant height. This equation is based on the relationship among average tree size, density and an indicator of productivity (i.e. (Goulding, 1972; Long & Shaw, 2005)). The remaining equations [4.3-4.10] relate the stand yield (expressed in several ways), to quadratic mean diameter, number of trees per hectare and dominant height.

$$d_g = b_0 \cdot N^{b_1} \cdot H_0^{b_2} \quad [4.2]$$

$$V = b_3 \cdot d_g^{b_4} \cdot H_0^{b_5} \cdot N^{b_6} \quad [4.3]$$

$$W = b_7 \cdot d_g^{b_8} \cdot H_0^{b_9} \cdot N^{b_{10}} \quad [4.4]$$

$$W_w = b_{11} \cdot d_g^{b_{12}} \cdot H_0^{b_{13}} \cdot N^{b_{14}} \quad [4.5]$$

$$W_c = b_{15} \cdot d_g^{b_{16}} \cdot H_0^{b_{17}} \cdot N^{b_{18}} \quad [4.6]$$

$$C = b_{19} \cdot d_g^{b_{20}} \cdot H_0^{b_{21}} \cdot N^{b_{22}} \quad [4.7]$$

$$E = b_{23} \cdot d_g^{b_{24}} \cdot H_0^{b_{25}} \cdot N^{b_{26}} \quad [4.8]$$

$$E_w = b_{27} \cdot d_g^{b_{28}} \cdot H_0^{b_{29}} \cdot N^{b_{30}} \quad [4.9]$$

$$UC = b_{31} \cdot d_g^{b_{32}} \cdot H_0^{b_{33}} \cdot N^{b_{34}} \quad [4.10]$$

Because of the existence of correlation between error components of the independent variables and dependent variables, the full information maximum likelihood method (*FIML*) was applied in the fitting process. The system of equations was fitted simultaneously by use of the MODEL procedure of the SAS/ETS® system (SAS Institute Inc, 2004). In the absence of convergence, equations were fitted by subgroups. The adjusted coefficient of determination and the root mean square error were used to check the accuracy of the models.

Mortality models were also considered for evaluating the non density dependent mortality. The model developed by García & Ruiz (2003) and Candy (1997) was used for *Eucalyptus globulus* [4.11] and *Eucalyptus nitens* [4.12] respectively.

The SDMDs were constructed by representing dominant height on the X-axis and the number of trees per hectare in logarithmic scale on the Y-axis. The quadratic mean diameter isolines were represented in the diagrams taking constant values for d_g and solving for N the equation [4.2]. The isolines for the equations [4.3-4.10] were represented in the diagrams solving d_g in each equation with [4.2], assuming constant values for each variable and solving for N. Mortality isolines were

also represented for each species. Since conversion between *t* and H_0 depends on site quality conditions, average observed *SI* values for each species were considered (Table 4.2).

$$N_1 = N_0 \cdot e^{-0,0281\cdot(t_1-t_0)} \quad [4.11]$$

$$N_1 = N_0 - N_0 \cdot \left(1 - e^{\left(-e^{A}\right)}\right),$$
$$\text{with } A = -8.6 + 0.1124 \cdot S + 0.1487 \cdot \left(\frac{t_0+t_1}{2}\right) - 0.0067 \cdot \left(\frac{t_0+t_1}{2}\right)^2 \quad [4.12]$$

where N_0 and N_1 are the number of trees per hectare at the age of t_0 and t_1 years respectively, and *S* (site index, m) is the dominant height at the age 15 years, estimated by the Candy (1997) model.

Two SDMDs were represented per species, one for carbon sequestration estimation (including additional isolines for quadratic mean diameter, carbon in aboveground biomass and organic soil, Hart-Becking index, mortality and sample plots for each species), and another for bioenergetic yield estimation (including additional isolines for usable cellulose, aboveground biomass energy, harvest limits and mortality).

4.2.5. Assessment of density limits and harvest limits

The relative spacing index (*HI*, %) was used to characterize the competence level at each development stage (Gadow & Hui, 1999), this was calculated as the ratio between the average distance among trees and the dominant height expressed as a percentage, with expression [4.13] and considering square spacing (Barrio Anta & Álvarez González, 2005; Barrio-Anta *et al.*, 2006; Castedo-Dorado *et al.*, 2009).

$$RS = \frac{10000}{H\sqrt{N}} \quad [4.13]$$

In order to determine the threshold density due to the self-thinning effect or full density curve (FDC, Jack & Long, 1996), the approach of Yoda *et al.* (1963) was used, considering the average crown biomass $\overline{W_c}$ (Zeide, 2005) as an indicator of crowding. This is because the stem occupies a negligible space with respect to the crown and roots, and therefore crowding is more closely related to crown biomass than to total aerial biomass.

$$\overline{W_c} \cdot N^{3/2} = K \quad [4.14]$$

As a convention, $\overline{W_c}$ at 1000 stems ha^{-1} (W_{x1000}) has been considered for defining the FDC (Sands & Landsberg, 2002; Almeida *et al.*, 2004), thus enabling determination of the value of the constant *K* that defines the curve. In this study, the average crown biomass (kg tree^{-1}) was plotted

against stand density to determine the maximum average crown biomass at 1000 stems ha^{-1} (W_{c1000}, kg tree^{-1}). The FDC equation was then derived from equation [4.14] by substituting K for $W_{c1000}\,1000^{3/2}$.

Although the -3/2 power law of self-thinning (Yoda *et al.* 1963) is assumed to be valid for all species and locations, some factors such as severe nutrient deficiencies (Lonsdale & Watkinson, 1982; Morris & Myerscough, 1991), climatic conditions and artificial shading (Aikman & Watkinson, 1980) may affect the trajectory of FDC. This implies that it is more appropriate to define a full density zone (FDZ), in which the probability of mortality due to the self-thinning effect is very high (Jack & Long, 1996). The lowest boundary limit of FDZ was determined as 70% of W_{c1000} (DeBell & Whitesell, 1988). Both limits of FDZ were represented in the diagrams by solving equation [4.14] for N.

Mechanized harvesting implies a size limitation due to the machinery used. Isolines of 10 and 20 cm of average stump diameter ($\overline{d}_{st}$, cm) were therefore represented in the diagrams. For this, a linear relationship between d_g and $\overline{d}$ was fitted for both species. $\overline{d}_{st}$ can be estimated accurately from the average diameter at breast height by use of a linear model (Diéguez-Aranda *et al.*, 2003). The model developed by Sánchez *et al*. (2004) was used to estimate *E. globulus* $\overline{d}_{st}$, and a similar model, developed with data collected in the plots established in the present study, was used to estimate *E. nitens* $\overline{d}_{st}$.

4.3. Results

4.3.1. Model parameters and additional relations

Results of the non-linear fit of equations [4.2-4.10], the coefficient estimates and the regression statistic values are shown in Table 4.3. All coefficients were significant at $p<0.05$, and the models accounted for more than 84% of the total variability in the quadratic mean diameter, and more than 94% of the total variability in productivity, equations [4.3-4.10].

As expected, the least accurate models were those predicting d_g, since the approach used in developing this type of diagram is to predict yield rather than increment, which makes them of little use, relative to dynamic growth models, for simulating a broad range of silvicultural regimes (García, 1994). With regard to the productivity equations, the C model was the least accurate, because the carbon in the soil organic layer is barely related to the independent variables considered, and is more closely related to time since last perturbation. The changes in total carbon in the aboveground biomass and in the soil organic layer over time are shown in Fig. 4.2.

Table 4.3. Non-linear regression coefficients and statistics obtained from simultaneous fitting of the system of 10 equations predicting quadratic mean diameter (d_g, cm), stand volume (V, m^3 ha^{-1}), total aerial biomass (W, Mg ha^{-1}), crown biomass (Wc, Mg ha^{-1}), total carbon in aboveground biomass and soil organic layer (C, Mg ha^{-1}), logging residue biomass (Ww, Mg ha^{-1}), total aerial energy without leaves (E, TJ ha^{-1}), logging residue energy (Ew, TJ ha^{-1}), usable cellulose production (UC, Mg ha^{-1}).

Equation	*Eucalyptus globulus* Parameter estimates				Adjusted R^2	RMSE
[4.2]	b_0=16.37744 (9.7711)	b_1=-0.38706 (0.0699)	b_2=0,.868226 (0.0858)		0.8480	3.1225
[4.3]	b_3=0.000064 (0.000018)	b_4=2.047556 (0.0504)	b_5=0.756605 (0.0482)	b_6=0.981972 (0.0235)	0.9950	17.8455
[4.4]	b_7= 0.000034 (5.775E-6)	b_8=2.132935 (0.0164)	b_9=0.732106 (0.0175)	b_{10}=0.97052 (0.0133)	0.9984	6.0913
[4.5]	b_{11}=0.000012 (1.79E-6)	b_{12}=2.457291 (0.0145)	b_{13}=0.077533 (0.0148)	b_{14}=1.036985 (0.0134)	0.9987	0.8934
[4.6]	b_{15}=0,.000021 (2.023E-6)	b_{16}=2.259985 (0.00986)	b_{17}=0.03626 (0.0109)	b_{18}=1.014372 (0.00884)	0.9992	0.4474
[4.7]	b_{19}=0.000073 (0.000029)	b_{20}=2.058496 (0.0528)	b_{21}=0.512052 (0.0602)	b_{22}=0.899273 (0.0426)	0.9911	6.9936
[4.8]	b_{23}=5.544E-7 (9.644E-8)	b_{24}=2.130056 (0.0168)	b_{25}=0.745888 (0.0179)	b_{26}=0.969512 (0.0136)	0.9984	0.1043
[4.9]	b_{27}=1.888E-7 (2.813E-8)	b_{28}=2.459492 (0.0146)	b_{29}=0.07792 (0.0149)	b_{30}=1.037102 (0.0135)	0.9987	0.0142
[4.10]	b_{31}=0.00001 (2.413E-6)	b_{32}=2.073132 (0.0228)	b_{33}=0.918631 (0.0247)	b_{34}=0.953745 (0.0180)	0.9974	3.3231
Equation	*Eucalyptus nitens* Parameter estimates				Adjusted R^2	RMSE
[4.2]	b_0=23.23792 (11.4785)	b_1=-0.34626 (0.0586)	b_2= 0.70549 (0.0728)		0.8437	3.0194
[4.3]	b_3=0.000068 (0.000017)	b_4=1.936645 (0.0752)	b_5=0.800026 (0.0628)	b_6=1,005736 (0.0252)	0.9956	14.9311
[4.4]	b_7=0.000025 (2.157E-6)	b_8=2.24867 (0.0140)	b_9=0.665185 (0.0128)	b_{10}=0.980934 (0.00781)	0.9987	4.2858
[4.5]	b_{11}=0.000026 (4.245E-6)	b_{12}=2.353757 (0.0326)	b_{13}=0.08703 (0.0265)	b_{14}=0.991036 (0.0168)	0.9960	1.5209
[4.6]	b_{15}= 2.714E-6 (5.996E-7)	b_{16}=2.756195 (0.0409)	b_{17}=0.180144 (0.0308)	b_{18}=1.002181 (0.0218)	0.9941	1.1395
[4.7]	b_{19}=0.000231 (1.97E-5)	b_{20}=1.902716 (0.1489)	b_{21}=0.485519 (0.1429)	b_{22}=0.834961 (0.1109)	0.9475	14.9173
[4.8]	b_{23}= 4.196E-7 (3.662E-8)	b_{24}=2.24873 (0.0140)	b_{25}=0.667739 (0.0128)	b_{26}=0.980905 (0.00783)	0.9987	0.0734
[4.9]	b_{27}=4.219E-7 (7.089E-8)	b_{28}=2.355724 (0.0341)	b_{29}=0.089347 (0.0277)	b_{30}=0.99033 (0.0175)	0.9957	0.0256
[4.10]	b_{31}=0.000016 (2.892E-6)	b_{32}=1.844636 (0.0275)	b_{33}=0.93374 (0.0290)	b_{34}=0.973451 (0.0137)	0.9956	3.4338

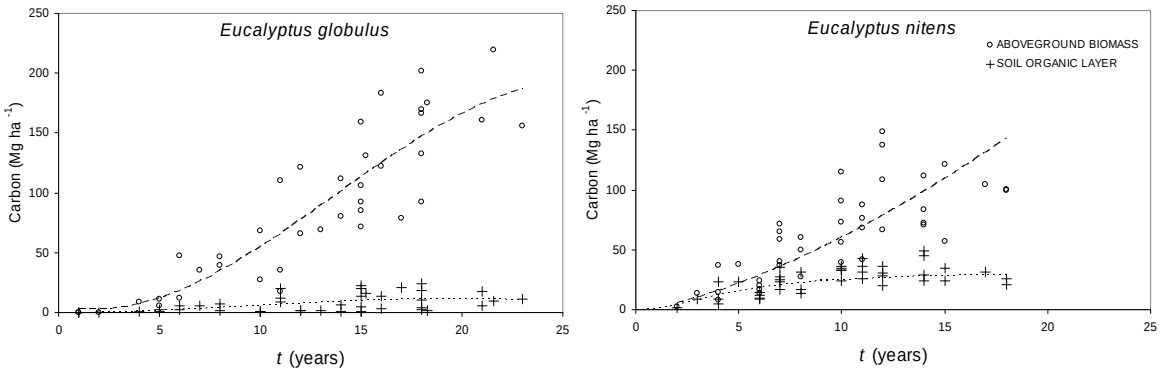

Figure 4.2. Carbon accumulation in aboveground biomass and soil organic layer over time (Mg ha^{-1}) for *Eucalyptus globulus* and *Eucalyptus nitens* plantations.

The dynamics of accumulation were quite different in both compartments (Fig. 4.2), while there was a high rate of accumulation of aboveground biomass carbon in both species (although dependent of site quality, Carbon at 8.3 Mg ha^{-1} yr^{-1} on average), the carbon in the litter tends to stabilize at 5-7 years in both species, and reaches values of Carbon at 10 and 25 Mg ha^{-1} at this age in *E. globulus* and *E. nitens* plantations.

The relation between the average crown biomass (kg tree^{-1}) and the number of trees per hectare for each plot and species, including several isolines of W_{c1000} is shown in Fig. 4.3. If we consider only the plots with low *IH* values and that probably undergo self-thinning (Lonsdale, 1990), the most reasonable values for the W_{c1000} are 60 kg tree^{-1} for *E. globulus* and 50 kg tree^{-1} for *E. nitens*. This indicates that *E. nitens* tolerates lower densities than *E. globulus*, which is consistent with previous findings for this species (Sims *et al.*, 1999a; Sims *et al.*, 2001). These values are equivalent to aboveground biomass of 360 kg tree^{-1} for *E. globulus* and 285 kg tree^{-1} for *E. nitens*, values similar to the self-thinning threshold defined to parameterize the 3-PG model for *E. globulus* in Australia (300 kg tree^{-1}, Sands & Landsberg, 2002).

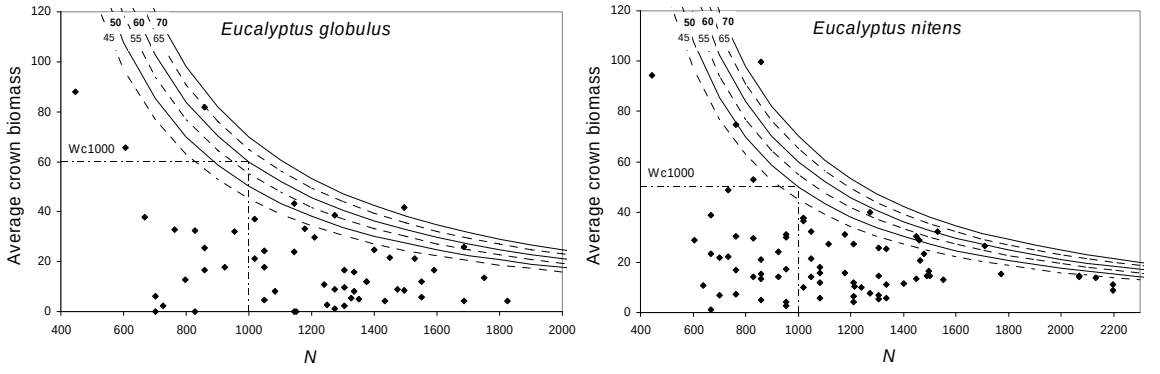

Figure 4.3. Relation between average crown biomass and number of stems per hectare (*N*) for *Eucalyptus globulus* and *Eucalyptus nitens* plantations: determination of self-thinning threshold.

The parameter values for the linear models of $\bar{d}$ estimation from d_g for each species are shown in Table 4.4. The model accuracy was very high for both species, allowing the incorporation of the harvesting limits for $\bar{d}_{st}$ of 10 and 20 cm.

Table 4.4. Equations for d_g estimation from $\bar{d}$ for *Eucalyptus globulus* and *Eucalyptus nitens* plantations.

	Equation	*Adjusted* R^2	*RMSE*
Eucalyptus globulus	$d_g = 1.0405 \cdot \bar{d} + 0.4067$	0.9954	0.5474
Eucalyptus nitens	$d_g = 1.0322 \cdot \bar{d} + 0.344$	0.9937	0.4365

4.3.2. Stand Density Management Diagrams

The SDMDs obtained for *E. globulus* and *E. nitens* are shown in Figs. 4.4-4.7. The isolines for quadratic mean diameter, carbon in biomass and organic soil, Hart-Becking index and mortality, as well as the positions of the sample plots are shown in Figs. 4.4 and 4.6. The isolines for usable cellulose, aboveground biomass energy, harvest limits and mortality are shown in Figs. 4.5 and 4.7. Although SDMDs provide useful graphic information about stand development stages, for more accurate estimation, equations [4.2-4.10] and the parameters shown in Table 4.3 must be used.

As can be seen in the SDMD, d_g decreases for a given H_0 as N increases because of increased competition for resources, which results in a smaller average tree size (Hamilton, 1969; Assmann, 1970; Curtis, 1970; Dean & Long, 1992). The pattern of d_g isolines is parallel to that observed for the harvest limits, and thus a threshold established for basal diameter would mean that a combination of initial planting density and dominant height at harvest would have to be considered.

For comparable stages of stand development (in terms of H_0 and N), *E. nitens* accumulates more carbon in biomass and soil organic layer, and this effect is partly attributable to the higher rate of accumulation of C in soil organic layer (Figs. 4.4 and 4.6).

The shape of biomass-related isolines (W, W_w, W_c, E and E_w) are more vertical than the volume-related isolines (V and UC) (Figs. 4.5 and 4.7). This indicates that biomass-related isolines are less sensitive to changes in N than volume related isolines. Moreover, for comparable stages of stand development, *E. nitens* has more energy in aboveground biomass and usable cellulose than *E. globulus*, because of faster diameter growth at comparable levels of N and H_0. These results are consistent with those of other studies for single stem crops of *E. nitens* at 2200 trees ha^{-1} (Sims *et al.*, 1999a).

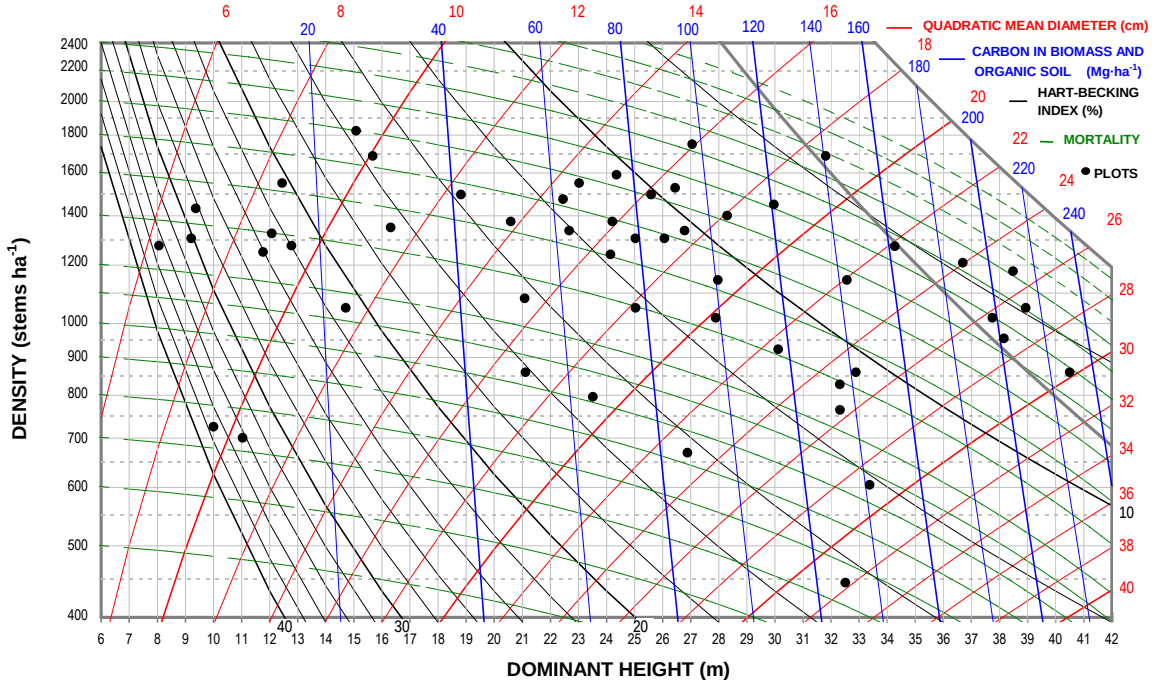

Figure 4.4. Stand Density Management Diagram for *Eucalyptus globulus* with isolines for: quadratic mean diameter, carbon in biomass and organic soil, Hart-Becking index, mortality and sample plots.

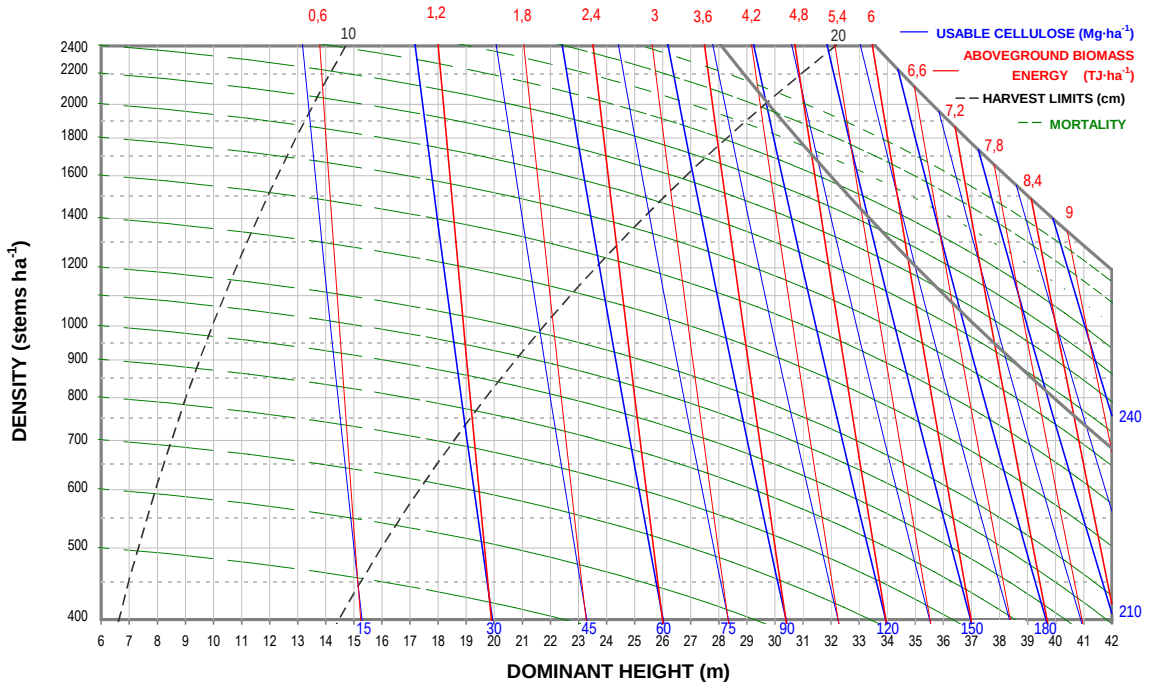

Figure 4.5. Stand Density Management Diagram for *Eucalyptus globulus* with isolines for: usable cellulose, aboveground biomass energy, harvest limits and mortality.

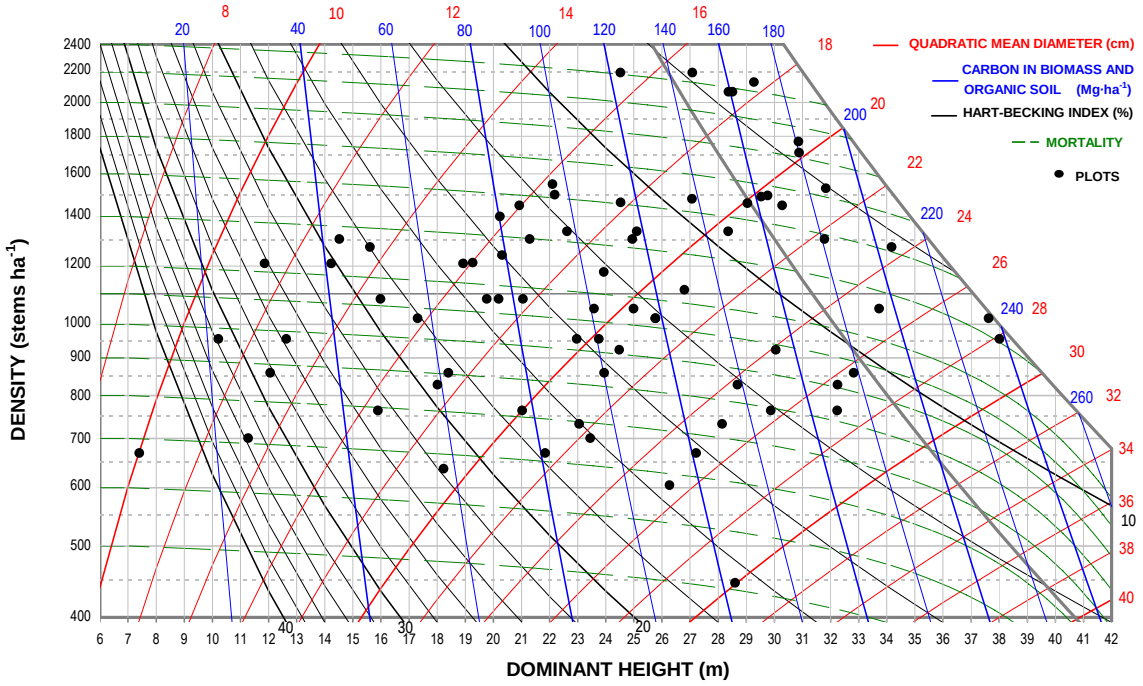

Figure 4.6. Stand Density Management Diagram for *Eucalyptus nitens* with isolines for: quadratic mean diameter, carbon in biomass and organic soil, Hart-Becking index, mortality and sample plots.

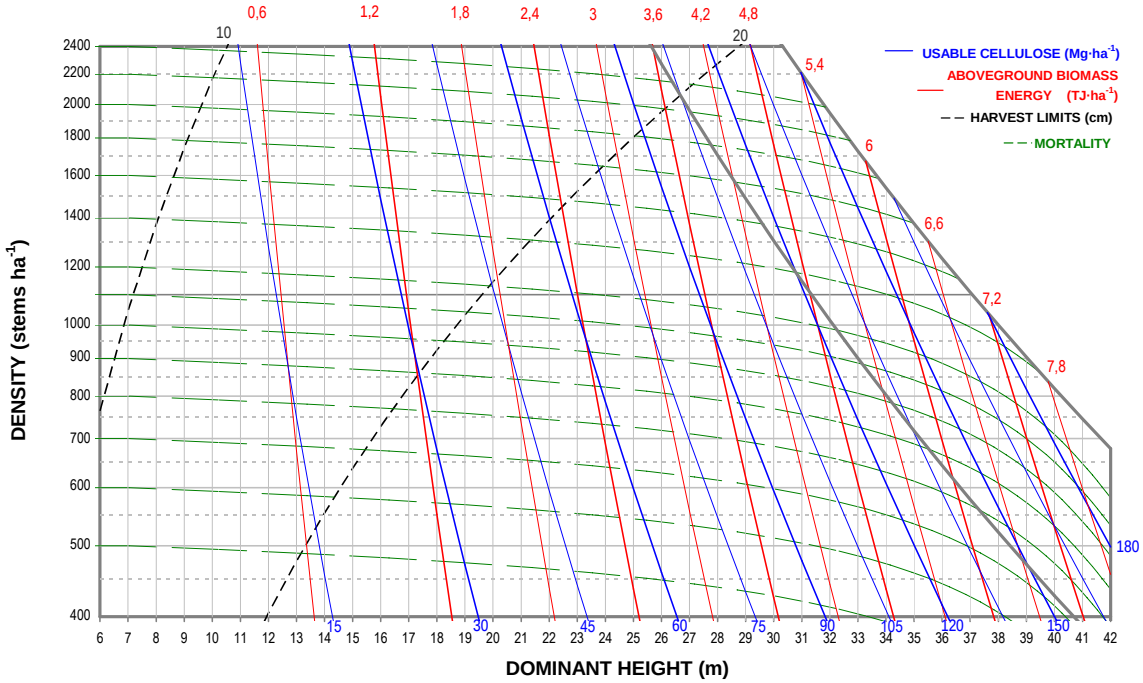

Figure 4.7. Stand Density Management Diagram for *Eucalyptus nitens* with isolines for: usable cellulose, aboveground biomass energy, harvest limits and mortality.

The changes in live-tree densities are shown in all the diagrams and allow estimation of expected natural mortality after planting in a scenario of no thinning, which is generally applied to woody crops destined for energy or fibre production. It must be considered that these trends were calculated by considering the average site index for both species and thus a site specific calculation using equations [4.11] and [4.12] is recommended if greater accuracy is sought. A high plantation density, together with longer rotations, results in high self-thinning mortality and unstable stems, as already corroborated (Sims *et al.*, 2001). As the initial density increases, the boundary of the FDZ is closer in terms of H_0 and therefore the management window is smaller. Although the relationship between the number of trees per hectare and the average tree size is a good indicator of density and therefore of competence, there are other factors that affect the self-thinning process, such as tree distribution and accumulation of gaps (Li *et al.*, 2000), which is not important when a regular square distribution is considered, but could be in the case of paired row layouts.

In the SDMD proposed here, several harvesting limits were represented. The $\bar{d}_{st}$ 10 cm limit defines the area in which a chip harvester may be more profitable. The $\bar{d}_{st}$ 10 and 20 cm limits define the area where a multi-cutter harvester may be more cost effective, whereas average basal diameters above $\bar{d}_{st}$ 20 cm would limit the choice to traditional single-stem harvesters.

Since all models depend on *N*, the production cost can be easily determined, and together with a correct estimate of site quality, will determine the time needed to obtain the desired products and therefore the economic profitability of the plantation.

4.3.3. Practical example of SDMD for determining energy production

SDMDs can be used to estimate production from a given stand development stage (*N* and H_0), or to estimate the minimum density that can provide a certain output for an average tree size. As a practical example, two silvicultural alternatives were simulated for each species, with initial densities of 2400 and 1600 stems ha^{-1}, representative of an energy option and a standard southern European pulp production target, respectively. The rotation time (*T*) was defined as the time that allows efficient harvesting of the crop by a multi-cutter harvester ($\bar{d}_{st}$ 20 cm) in the case of the energy target for an average site index. For the standard pulp silviculture, stand growth was simulated until one of the two species reached the FDZ area, for which *E. nitens* occurred at a dominant height of 29 m. The stand development stages at the time of harvest and rotation time for the average site index are shown for both alternatives and species (Table 4.5). The predicted d_g at the end of rotation and yield values for *V*, *W*, W_w, W_c, *C*, *E*, E_w and *UC* for the two silvicultural alternatives are also shown.

The total aboveground biomass yield for all fractions ranged between 13.9 and 14.6 Mg ha^{-1} yr^{-1} for *E. globulus* and between 20.4 and 21.5 Mg ha^{-1} yr^{-1} for *E. nitens* (Table 4.5). The mean annual increment in terms of biomass was only marginally higher for the high density silviculture, although the rotation age was also shorter in this case. The values for the expected yield in terms

of energy or usable cellulose were higher for *E. nitens*, although direct comparison is difficult, as the species grow in different areas.

Table 4.5. Predicted yield for the two silvicultural alternatives considered.

	N_0=2400				N_0=1600			
	Eucalyptus globulus		*Eucalyptus nitens*		*Eucalyptus globulus*		*Eucalyptus nitens*	
N_1 (stems ha^{-1})	1700		2100		925		1300	
H_{01} (m)	27.6		27.2		28.5		28.7	
T (years)	14		11		15		12	
d_g (cm)	16.7		16.8		20.3		20.2	
Yield units	ha^{-1}	ha^{-1} yr^{-1}	ha^{-1}	ha^{-1} yr^{-1}	ha^{-1}	ha^{-1} yr^{-1}	ha^{-1}	ha^{-1} yr^{-1}
V (m^3)	356.6	25.5	503.0	45.7	356.3	23.8	489.7	40.8
W (Mg)	204.1	14.6	236.3	21.5	208.2	13.9	244.6	20.4
W_w (Mg)	33.5	2.4	52.9	4.8	34.6	2.3	53.9	4.5
W_c (Mg)	24.8	1.8	25.5	2.3	24.9	1.7	28.0	2.3
C (Mg)	101.1	7.2	148.4	13.5	104.2	6.9	151.9	12.7
E (TJ)	3.430	0.245	4.000	0.364	3.501	0.233	4.141	0.345
E_w (TJ)	0.531	0.038	0.865	0.079	0.549	0.037	0.882	0.074
UC (Mg)	83.2	5.9	110.9	10.1	85.0	5.7	108.4	9.0

4.4. Discussion

4.4.1. Model performance and limitations

The model presented here represents a system of related equations that enable accurate estimation of crop yield in terms of oven dry biomass, total energy, usable cellulose and other variables, and thus provides a powerful tool for decision making as regards woody crops of the species studied. The diagrams show the crop development as dominant height increases, and are therefore independent of age and valid for applying to different breeding materials and sites for each species, provided the change in dominant height with age is known in each case. These empirical statistically based tools can also be combined with process-based models if the detailed information required in this case is available.

Woody biomass can be converted via combustion, gasification, pyrolysis and fermentation, and the energy recovered depends on the conversion technology (McKendry, 2002). Although the energy obtained from *LHV* is a theoretical value that can only be achieved at 0% moisture, the information obtained from the diagrams, particularly the share of logging residues, and the proportion of cellulose or stem volume, is useful for determining the suitability of the biomass produced for subsequent processing.

The models provided here are only valid for single stem rotations, which is an important constraint to be considered. However, this is not a key limitation in the case of *E. nitens*, because some studies have shown the poor coppicing ability of *E. nitens* (Sims *et al.*, 2001; Little & Gardner, 2003), at least for the breeding materials currently in use. Changes in growth patterns and relationships among stand variables after coppicing have been shown, particularly the change in basal area derived from the sprout number per stool (Sims *et al.*, 1999b). It is therefore necessary to obtain a similar model for *E. globulus* coppice stands in order to assess the long term productivity.

One of the main advantages of the models provided in this paper is the possibility of assessing the economic profitability of SRC with eucalypts, because many variables are needed to calculate the cash flow throughout the rotation. Most production costs, from establishment to delivery, can be calculated as they are density-dependent, as is the case of plantation, localized fertilization and weed control. Plant material may account for up to 65% of establishment costs and any advantage gained by high planting rates may be outweighed by increasing costs (Mitchell *et al.*, 1999). Stand harvesting costs constitute a major portion of total production costs, and may have effects as important as those of stand establishment costs (Whitesell *et al.*, 1992). Harvest operations represent up to 70% of the cost in the overall supply chain, and therefore less frequent harvesting reduces the cost of biomass production per unit (Mitchell *et al.*, 1999). Moreover, average tree size is the most important factor in harvesting costs (Whitesell *et al.*, 1992). The information provided in the diagrams, particularly the average basal diameter, is also essential for deciding what type of harvesting to carry out, i.e. whole stem harvesting or chip harvesting.

4.4.2. Carbon sequestration

The diagrams obtained in this paper and the fitted equations constitute a powerful tool for assessing carbon sequestration in eucalypts grown in SRF. The isolines plotted in the diagrams range between 20 and 200 Mg ha^{-1} for carbon in aboveground biomass and litter. Since no information was obtained for root biomass it was not possible to assess sequestration in this compartment, which is an important consideration because root biomass is usually left in situ after the crop is harvested.

The values for carbon in litter show greater accumulation of C in the litter resulting from *E. nitens* than in *E. globulus* crops. Net production in a forest should be assessed as the sum of biomass accumulation and annual litter production, in which *E. globulus* plantations of density 4167 stems ha^{-1} may reach 13.4 Mg ha^{-1} yr^{-1}, and as much as 10-20% of the total biomass production at age 3 years (Toky & Ramakrishnan, 1983). Although this litter can hardly be used for energy production, estimation of the quantity is important for estimating carbon accumulation and nutrient cycle return. Although the soil mineral layer was not considered here, carbon accumulation in this pool is very important because the lifespan of the carbon is longer than in biomass and in the soil organic layer (Romanya *et al.*, 2000).

4.4.3. Comparison of data from other studies

The biomass yields (Table 4.5) are higher than the 1-9 Mg ha^{-1} yr^{-1} reported for 4-year-old *E. globulus* planted at a density of 2196 stems ha^{-1} (Cromer *et al.*, 1975), but lower than the 24 Mg ha^{-1} yr^{-1} observed in 3-year-old plantations of the same species in New Zealand, established at a density of 4167 stems ha^{-1} and irrigated with effluent. No irrigated stands yielded as much as 19.3 Mg ha^{-1} yr^{-1} (Guo *et al.*, 2002). *E. globulus* planted at densities of 20000, 30000 and 40000 stems ha^{-1} in Portugal (Pereira *et al.*, 1994) yielded 16, 21 and 19 Mg ha^{-1} yr^{-1} after 2 years. The general effect is therefore a similar average yield (obtained early on because of the initial high stocking density) but with a decrease in the average tree size (Dickmann, 2006). Referenced yield data for *E. nitens* in SRF are scarce and values are very low compared with those observed in the present study. Sims *et al.* (2001) reported 3-7.3 Mg ha^{-1} yr^{-1} for single stems established at a density of 5000 stems ha^{-1} and first coppice rotation, respectively, with very low survival. As regards other *Eucalyptus* species, Wise & Pitman (1981) collected yield data for several *Eucalyptus* species in Australia, and observed values of between 11 and 16 Mg ha^{-1} yr^{-1}. Sachs *et al.* (1980) reported *Eucalyptus* yields as high as 40 Mg ha^{-1} yr^{-1} in a wide range of sites. The average yield for *Eucalyptus* species in a single stem rotation at a density of 2200 trees ha^{-1} reported by Sims *et al.* (1999a) ranged between 9.6 and 15.5 Mg ha^{-1} yr^{-1}, which are the highest reported values for the species, with high survival rates and larger average tree size. The values observed here were similar or higher and were determined from commercial sized plots with conservative assumptions for site quality.

With regard to energy production at the end of rotation, the predicted values ranged between 3.4-3.5 and 4.0-4.1 TJ ha^{-1} for *E. globulus* and *E. nitens* respectively, well above the values reported for poplar (173-259 GJ ha^{-1}; 10-15 Mg ha^{-1} yr^{-1}) and willow (187-280 GJ ha^{-1}; 10-15 Mg ha^{-1} yr^{-1}) grown as short rotation woody crops. Mean values ranged from 245 to 345 GJ ha^{-1} yr^{-1}, still far from the average range for eucalypt plantations in Aracruz (450 to 650 GJ ha^{-1} yr^{-1}, (Moreira, 2006)). The annual logging residue energy yield was 33-35 and 53-54 GJ ha^{-1} yr^{-1} for *E. globulus* and *E. nitens* respectively, similar to the 65 GJ ha^{-1} yr^{-1} estimated for both species together, also in northern Spain (Pérez *et al.*, 2008).

Rotations simulated for the bioenergy alternative in this paper are longer than the average considered in SRC, but there are positive effects of this practice. If wood is the main biomass compartment desired, longer rotations provide higher wood:bark ratios, as shown in Fig. 4.8 for the data used in the present study. The wood:bark ratio can reach up to 80% in *E. globulus* at 10 years (Guo *et al.*, 2002), but data reported for *E. nitens* at 3 years indicate a rather low value of 45% (Sims *et al.*, 1999a). Moreover, longer rotations result in larger average tree size, and therefore harvest machinery is better able to discriminate between leaves and other tree fractions, which reduces the nutrient exports and ash production. Bark and leaves contain the highest amounts of ash in all aboveground biomass compartments, with reported values of 1.5-2 times (Pérez *et al.*, 2008), and even 10 times (Ragland & Aerts, 1991) the wood ash content; this is an important

problem if combustion is the chosen biomass transformation, as the high ash content can damage the boilers used. Longer rotations are also a good option if the goal is product flexibility (Dickmann, 2006). The results of the present study for the single stem rotation showed that 50% increments in initial density result in only marginal increases in yield as well as decreases in rotation, as previously observed (Whitesell *et al.*, 1992).

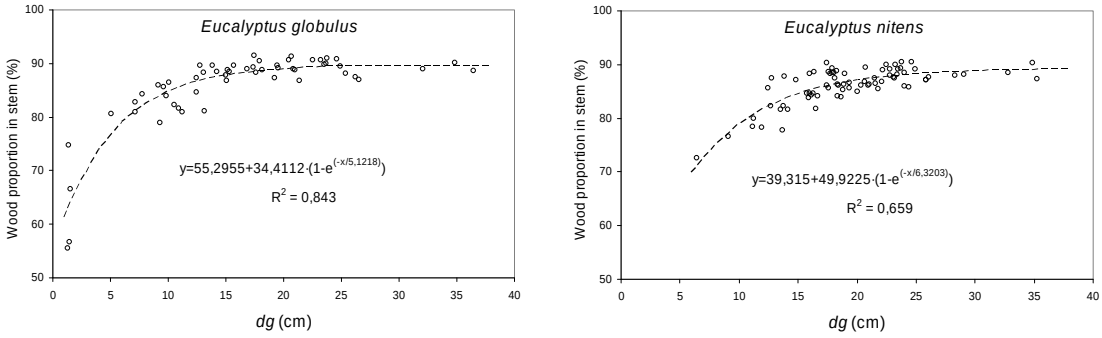

Figure 4.8. Wood proportion in the stem in relation to quadratic mean diameter for both species.

4.5. Conclusions

Management tools for estimation of bioenergy production and carbon sequestration in single stem *Eucalyptus* plantations are presented in the form of dynamic SDMD. These models allow yield simulations for a range of initial density and rotation age options and provide information about the value of the crops, by use of input data usually available from routine forest inventories. The observed yields for *Eucalyptus globulus* and *Eucalyptus nitens* planted at densities of 1600-2400 stems ha^{-1} in single stem rotation are similar to those observed for the higher densities usually used in SRF. *Eucalyptus nitens* displayed faster diameter growth than *Eucalyptus globulus* planted at similar densities. Mean values of potential annual energy production from total biomass, except leaves, for average site indices for both species in northern Spain ranged between 233-245 and 345-364 GJ ha^{-1} yr^{-1} for *Eucalyptus globulus* and *Eucalyptus nitens*, respectively. The options simulated in this paper display good product flexibility and would provide high wood:bark ratios.

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Chapter V

Application of calorimetry and thermal analysis to study the stabilization of soil organic matter in afforested soils

5. Application of calorimetry and thermal analysis to study the stabilization of soil organic matter in afforested soils

Abstract

The study of soil organic matter dynamics requires highly reproducible and accurate techniques for application to large numbers of samples. This is especially important for modelling changes in soil organic matter in relation to land use changes, as required by the Kyoto protocol. The main objective of this study was to apply calorimetry and thermal analysis, as novel techniques to elucidate how afforestation affects the nature of SOM and soil microbial metabolism. The techniques were applied to study the SOM dynamics in two afforested stands differing in the tree species used, *Pinus radiata* and *Eucalyptus globulus*, established on pastures in a humid temperate region. The application of differential scanning calorimetry and solid state nuclear magnetic resonance revealed that the soil organic matter was constituted by carbohydrates, carbonyl/carboxyl groups, aliphatic components and aromatic carbon in the first years of the rotation. All these fractions became degraded after afforestation. The degradation was monitored by calorespirometry, which provided the calorespirometric ratio of the soil basal metabolism, together with the active biomass and the metabolic quotient. These indexes proved to be sensitive parameters that provided information about changes in the pattern of microbial metabolism in response to changes in the nature and redox state of the carbon substrates, demonstrating degradation of the aromatic and aliphatic organic matter fraction. The techniques were able to distinguish differences in soil organic matter dynamics in the two types of stands, attributable to the different development of understory vegetation and litter composition.

Keywords: calorimetry, thermal analysis, afforestation, SOM, biodegradation.

5.1. Introduction

Changes in land cover and certain types of land management (such as intensive forestry and agriculture), wildfires, and drainage alter the pools and turnover rates of soil organic carbon (SOC). Since soils represent one of the largest reservoirs of organic C on a global scale, huge amounts of CO_2 and other greenhouse gases are being transferred from the soil to the atmosphere as a consequence of such activities. Although important advances have been made in research on this

topic in the last decade, current knowledge of the mechanisms involved in the dynamics of soil organic matter (SOM) still does not enable prediction of the ecosystem response to such perturbations.

A substantial part of SOM can be restored by means of conversion of arable land to forest land. For this reason, afforestation is a major strategy included in the Kyoto Protocol on climate change, because of the capacity of forests to restore C in biomass and soils. Although these measures contribute to C sequestration in biomass, the impact on soil C is less clear. Since C sequestration in soils is influenced by numerous factors, such as previous land use, climate and tree species (Paul *et al.*, 2002), it is difficult to establish a common protocol predicting the changes in C in relation to afforestation. Thus, different studies have detected important decreases in SOM during the first years after afforestation as a consequence of the increased C mineralization (Turner & Lambert, 2000; Davis & Condron, 2002). The loss of SOM is attributed to the reduced inputs of labile organic compounds to the mineral soil, which creates an imbalance between inputs of C and respired C (Chen *et al.*, 2000; Saggar *et al.*, 2001), indicating the crucial role of microorganism activity in SOM dynamics. One of the most important difficulties in modelling the soil C sequestration that occurs after afforestation is the complexity of the SOM dynamics, resulting from simultaneous mechanisms, such as molecular recalcitrance, spatial inaccessibility of the decomposer and stabilization by clays and ions (e.g. Sollins *et al.*, 1996; von Lützow *et al.*, 2006). Moreover, it is known that the degradation of recalcitrant OM can easily take place in upper horizons (80% of the SOM in most soils), in which other stabilization mechanisms, such as the spatial inaccessibility of microorganism to SOM and enzymes or interaction with mineral surfaces and metal ions, are less active than in deeper soil horizons (von Lützow *et al.*, 2006). As a consequence, SOM turnover is usually rapid in the upper horizons, but takes longer at greater depths (Fierer *et al.*, 2003; Goberna *et al.*, 2006). For all of these reasons, evaluation of the effects of afforestation on C balance and SOM turnover must be made by characterization of the composition and the stability of SOM substances and by the use of indicators of microbial activity.

Analytical techniques such as solid-state 13 C CP-MAS NMR, Fourier transform infrared spectroscopy and pyrolysis/GC-MS are widely used to characterize the structure and composition of SOM. In addition, different fractionation methods have been developed in the last decade to evaluate the relative abundance of labile and recalcitrant forms of C (e.g von Lützow *et al.*, 2007). Same examples of such fractionation approaches include chemical and physical procedures (Sohi *et al.*, 2001; Rovira & Vallejo, 2002; Marriott & Wander, 2006). Moreover, microbial biomarkers and compound-specific stable isotope and radiocarbon analysis enable differentiation of functional fractions (Rethemeyer *et al.*, 2004; Brovkin *et al.*, 2008). All of these strategies constitute valuable tools that have provided important insights into SOM dynamics. However, some studies on dynamics of SOM, especially those focussed on modelling, require simple techniques that could be used for large number of samples, while at the same time offering a high degree of reproducibility and accuracy. In this sense, calorimetry and thermal techniques avoid the need for some of these long procedures, and provide quantitative assessment of SOM turnover and stabilization.

In recent years, calorimetry and thermal analysis have been use to meet the increasing demand for rapid and more reproducible assessment of SOM properties (Barros *et al.*, 2007). Differential Scanning Calorimetry (DSC) provides information about thermal properties of SOM in relation to its composition and stabilization. These techniques are based on the different temperatures of exothermic decomposition of carbohydrates and carboxyl groups, aliphatic substances and more refractory aromatic C. Thus, DSC has been used to offer a relatively rapid and simple analytical method to study SOM fractions of soils subjected to different types of management or perturbations, such as wildfires (De la Rosa *et al.*, 2008; Duguy & Rovira, 2010), land uses changes (Lopez-Capel *et al.*, 2005; Salgado *et al.*, 2010) and intensive tillage (Plante *et al.*, 2005). The combined use of DSC and other analytical techniques enables better characterization of SOM. Thus, recent studies have combined DSC with e.g. microbial activity indicators (Marinari *et al.*, 2010), SOM chemical and physical fractionation (Dell'Abate *et al.,* 2002; Lopez-Capel *et al.,* 2005; Plante *et al.,* 2005), changes in isotopic C (Kuzyakov *et al.*, 2006; Dorodnikov *et al.*, 2007), pyrolysis/GC-MS (De la Rosa *et al.*, 2008), Fourier transform infrared spectroscopy (Marinari *et al.*, 2010) and NMR (Lopez-Capel *et al.*, 2005; Barros *et al.*, 2011).

In the study of the relationships between microbial activity and SOM stabilization, certain indicators such as the microbial biomass pool, the microbial quotient (microbial biomass/SOC) and metabolic quotient (soil respiration/microbial biomass, qCO_2) have been used to assess substrate degradability and the degree of substrate limitation for soil microbes (Dilly & Munch, 1998; Bastida *et al.*, 2008). Thus, the application of these parameters provides information about how rapidly organic substrates are metabolized.

Soil microbial metabolism can also be measured by isothermal calorimetry, which has been used to study metabolism in microorganisms, animals and plants (Hansen *et al.*, 2002). Despite the different advantages of this technique, its application to soils is still quite limited (Barros *et al.*, 2007; Barros *et al.*, 2011). Since this technique is not invasive, it can improve the accuracy and reproducibility of the determinations. It is also a simple, sensitive and reliable technique, which reduces the problems derived from the lengthy handling procedures, and enables continuous monitoring of soil microbial activity.

Thus, calorimetry provides data on microbial biomass (Sparling, 1983) and simultaneous measurement of metabolic heat rate (Φ) and soil respiration, data that are well correlated with those measured by other more traditional techniques (Sparling, 1981; Critter *et al.*, 2004b). Moreover this technique can also provide quantitative indices of microbial metabolism (such as the heat released per unit of microbial biomass), which provide information about the efficiency of carbon utilization by soil microorganisms (Barros & Feijóo, 2003; Zheng *et al.*, 2009). Another useful and novel index obtained by this technique is the calorespirometric ratio (Φ/RCO_2), which provides information about the redox state of substrates being metabolized by the microorganisms, and the efficiency of conversion of substrate carbon into living cells in processes associated with microbial biomass gain (Hansen *et al.*, 2004; Wadsö *et al.*, 2004). This parameter has been successfully used in plants and insect studies (Acar *et al.*, 2004; Summers *et al.*, 2009), but

determination of the Φ/RCO_2, was not reported for basal metabolism in soils until recently (Barros *et al.*, 2011). The latter study shows that measurements of this ratio can provide information on the nature of the organic substrates being degraded in the soil and may therefore contribute to improving our knowledge about SOM turnover.

The main objective of this study was to use calorimetry and thermal analysis to elucidate how afforestation affects the nature and dynamics of SOM in a humid temperate region, where the SOM dynamics are particularly rapid. DSC was used in combination with ^{13}C CP-MAS NMR to study the changes in SOM throughout the rotation, after afforestation. The effect of changes in SOM composition on microbial metabolism was studied by isothermal calorimetry. This should contribute to our understanding of the SOM stabilization processes by providing new insight into SOM properties.

5.2. Material and Methods

5.2.1. Stands selected and sampling

The study was carried out in NW Spain (Lugo). All stands were located on former pastures, in which low intensive management was applied over many years, and which have recently been afforested with *E. globulus* and *P. radiata*. The stands were located no more than 30 km apart and they had comparable land use history prior to afforestation. Similar forest management was carried out in each type of forest plantation. To ascertain that all sites were similar as regards soil type and land use, selection of the study sites was based on direct observation of the terrain of adjacent pastures, representing the average tendency for each species reported in Chapter II, as well as consultations with local landowners.

The ages of the *Eucalyptus globulus* stands were 1, 5 and 18 yr, and of the *Pinus radiata* stands 3, 13, 28, 35 and 40 yr, which corresponded to different stages of the rotation of these species in the region (15-18 yr in the former and 35-40 yr in the latter). These stages were: (i) establishment (stands 1E and 3P), which in the eucalypts and pine stands are 1 and 3 yr of age, respectively; (ii) young stages, immediately after canopy closure (5E and 13P); (iii) mature plantations (28 and 35P), and (iv) end of rotation (18E and 40P).

The 20 year annual average rainfall of the area is 1158 mm, and the temperature, 15.1 ºC. The wettest month is November, with an average rainfall of 139 mm, and the driest August, with 45 mm. The lowest mean monthly temperature 9.7 ºC occurs in February, and the highest 19.1 ºC, in August. The soils were developed from schist and quartzite, and were classified as Alumi-humic Umbrisol (IUSS Working Group WRB, 2006). The soil has a loam or sandy loam texture and is well drained. The A horizon is rich in organic matter and strongly acidic (Table 5.1). The soil humidity

and temperature regimes are Udic (mean period with partial drought, 1 month) and Mesic (mean frost-free period, 10 months), respectively.

Table 5.1. Characteristics of stands and selected properties of the soil samples (0-5 cm), and organic layer.

Tree Species	Sample	Age (yr)	Stocking (tree ha^{-1})	Tree height (m)	Organic Layer (Mg ha^{-1})	pH	Clay (%)	Soil Texture
Eucalyptus globulus	1E	1	1146	1.3	0.00	5.88	13	Loam
	5E	5	1432	7.7	2.70	5.57	15	Sandy Loam
	18E	18	1146	23.0	10.14	4.95	11	Loam
Pinus radiata	3P	3	668	1.91	0.31	5.82	16	Silty Loam
	13P	13	891	15.15	30.96	5.62	17	Loam
	28P	28	859	20.46	56.22	5.32	5	Sandy Loam
	35P	30	923	19.54	49.07	5.36	6	Sandy Loam
	40P	40	923	25.05	69.32	4.69	17	Silty Loam

For soil sampling, a 50 m x 50 m plot was selected within the stand, at a distance of more than 30 m from the edge of the stand. In each plot, forest floor and mineral soil samples were taken at 8 randomly distributed points. The organic horizons were collected with the aid of a frame (25 cm x 25 cm). Sub-samples of the mineral soil layer (0-5 cm) were collected with a steel corer, and were combined to form one bulk sample per plot. Site preparation for forest establishment consisted of ripping, and no fertilization, tillage or weed control was carried out in the plantations.

5.2.2. General soil properties

The pH of the soil was measured in 0.1 M KCl with a glass electrode. Total C and N were analyzed with a LECO Elemental analyzer (LECO TruSpec CHNS). Soil particle analysis was performed by laser diffractometry, with a Mastersizer 2000 diffractometer.

5.2.3. Thermal analysis

The soil was dried and gently ground in an agate mortar for DSC measurements (DSC Q100 TA instruments). These experiments were conducted at a heating rate of 10°C min^{-1} under a flux of dry air from 20 to 600 ºC, as previously described (Dell'Abate *et al.*, 2000)**.** The direct integral of the exothermic DSC curves under the flux of dried air with respect to zero gives the heat of combustion of the soil in kJ g^{-1}. All measurements were made on dry weight basis.

5.2.4. Calorimetric measurements

Soil samples were sieved (2 mm) and stored at 4 ºC in polyethylene bags. Prior to calorimetric experiments, samples were brought to 25% moisture content, and incubated for about 24 hours at the temperature of the calorimetric measurements, 25ºC.

The soil basal metabolism was monitored in a TAM 2277 calorimeter (TA Instruments), which is a very sensitive heat conduction calorimeter with 3 calorimeter channels. Each channel has two calorimeter ampoules: one for the sample and the other for reference samples. The calorimeter was statically calibrated for the soil measurements at an amplifier setting of 300 microwatts (μW). Each soil sample was prepared for calorimetric measurements after equilibrating at 25 ºC by weighing 1.5 g into three 4 ml stainless steal ampoules; the open ampoules were then left, together with a vial containing water, in a sealed polyethylene bag, for 48 hours. This treatment allows the soil metabolism to equilibrate from the storage temperature, 4ºC, to the measurement temperature, 25ºC, and allows the samples to reach vapour equilibrium. A small vial containing 0.2 mL of 0.4M NaOH was then placed in one of the sample ampoules. The three sample ampoules were then closed and placed in the calorimeter at the same time, together with the reference ampoules filled with silica sand.

The calorimetric channels with soil measure basal metabolic heat rate, Φ continuously in microwatts, μW or μJ s^{-1}, while the channel with NaOH measures the soil basal metabolic heat rate plus the heat rate from reaction between metabolic CO_2 and NaOH, both of which are exothermic. The enthalpy of the CO_2 reaction is -108.5 kJ mol^{-1} at this concentration of NaOH (Criddle *et al.*, 1991; Russell *et al.*, 2006). Φ was measured at 3 minute intervals for between 20 and 48 hours. At the end of the experiment, the NaOH was removed from the calorimeter and the sample resealed and replaced in the calorimeter to check the basal metabolic rate of the soil sample containing the NaOH. This procedure enables the reproducibility of the registered basal metabolism in the three soil samples to be checked.

The metabolic heat rate, Φ, the rate of CO_2 production, RCO_2, and the ratio of metabolic heat rate to CO_2 rate, Φ/RCO_2, were calculated from the tabulated Φ data by averaging the Φ values from the two channels with only soil and subtracting the values from the Φ measured from the soil sample with NaOH. The results obtained are the tabulated Φ data for the reaction between the CO_2 and the NaOH, which were then divided by the enthalpy change (-108.5 kJ mol^{-1}) for the reaction between the NaOH and the CO_2, to give RCO_2 at each data point in mol CO_2 per second. The quotient between the basal metabolic Φ and the tabulated RCO_2 values yields Φ/RCO_2. The Φ, RCO_2, and Φ/RCO_2 can be reported quantitatively as the average values of the tabulated data. The standard deviation of the tabulated data provides information about the variability in these values. The quantitative RCO_2 was related to the active biomass to give the metabolic quotient, qCO_2. Soil active biomass was calculated by the Sparling method (Sparling, 1983) and related to the soil C content to give the ratio of microorganisms to soil carbon, C_{mic}-C.

5.2.5. Solid state ^{13}C CP-MAS NMR

Solid NMR experiments were performed at 298 K in a 17.6 T Varian Inova-750 spectrometer (operating at 750 MHz proton frequency) equipped with a T3 Varian solid probe (Agilent, Inc, USA). Solid NMR samples were prepared in 3.2 mm rotors with an effective sample capacity of 22 μL, which corresponds to approximately 30 mg of the powdered sample. Carbon chemical shifts were referred to the carbon methylene signal of solid adamantine, at 28.92 ppm. This sample was also used to calibrate the 1D CP-MAS experiments.

Cross Polarization Magic Angle Spinning (1D CP-MAS) experiments were carried out with the samples, under the following conditions: the inter-scan delay was set at 0.5 s, the number of scans was 100000 and the MAS rate was 15 kHz. Heteronuclear decoupling during acquisition of the FID was performed with Spinal-64, at a proton field strength of 70 kHz. The cross polarization time was set at 1 ms. During cross polarization, the field strength of the proton pulse was held constant at 75 kHz, and that of the ^{13}C pulse was linearly ramped with a 20 kHz ramp near the matching sideband. The NMR spectra were processed and the area of the signals was quantified with MestreNova software (Mestrelab Research inc).

5.2.6. Statistical analysis

The quantitative indices are given as the average of three replicates and the standard deviation. The data were compared by graphical analysis. All the correlations were significant at $p<0.05$.

5.3. Results

5.3.1. Changes in the SOM in the afforested soils

The changes in the soil organic layer and SOC in the uppermost, superficial mineral soil layer (0-5 cm) throughout the rotation period for each species (15 yr and 35-40 yr are the usual rotation periods for *E. globulus* and *P. radiata* in the region) are shown in Table 5.1, and as expected, litter accumulation increased with age in both types of stands, although the process was much faster in the pine stands than in the eucalyptus stands. In the *P. radiata* stands litter accumulation started earlier, and the amount accumulated at the end of the rotation was 7 times higher than in the latter.

The mineral soils of both plantations were subjected to large decreases in SOC throughout the rotation. The soils under pines lost more SOC (maximum recorded loss, 70% after 30 yr) and over a longer time that the soil under eucalyptus (maximum recorded, 50% after 5 yr). At the end of the rotation both soils partially recovered their SOC contents. In the pine chronosequence, the C gain

in mineral soil occurred when the litter accumulation was close to steady state. The C/N ratio decreased in the same way as SOC (Table 5.2), and reached values of around 10 in the soils with the highest SOC losses.

Table 5.2. SOC, SON and SOM contents of the samples, together with the carbon to nitrogen ratio (C/N) and the microbial active biomass.

Tree Species	Samples	C (%)	N (%)	C/N	SOM(1) (%)	Microbial Biomass (µg CX g^{-1})
E. globulus	1E	11.0	0.83	13.2	24.07±0.45	553±16
	5E	5.2	0.56	9.3	12.53±0.80	387±28
	18E	9.1	0.75	12.2	17.37±0.46	270±14
P. radiata	3P	12.4	0.88	14.1	27.22±1.77	767±98
	13P	6.5	0.47	13.9	16.97±0.19	475±97
	28P	3.7	0.35	10.5	5.26 ± 0.10	273±19
	35P	4.4	0.44	10.0	9.41±0.18	69±12
	40P	6.7	0.46	14.7	13.00±0.33	183±45

(1) Soil organic matter.

5.3.2. SOM composition in the mineral soil horizon: DSC and NMR

The heat of combustion (Q) of the soil samples, in Joules per gram of soil, determined by direct integration of DSC curves (Fig. 5.1), and the maximum heat of combustion temperatures for SOM are shown in Table 5.3. Q was positively correlated with SOC and SOM (R^2 = 0.83 p< 0.001; R^2 = 0.88 p<0.001 respectively), reflecting the direct connection between Q and the SOM content of the samples. Such correlations have been reported in previous studies (Barros *et al.*, 2008) and indicate that the heat of combustion of the soil, determined by DSC, is only a function of the SOC and SOM contents when given in Joules per soil mass. Thus, the largest areas limited by the DSC curves for samples 1E and 3P was related to the higher SOM contents, whereas the small areas limited by the DSC curves for samples 5E, 28P and 35P corresponded to the lower SOM contents.

Typical DSC curves of the soil samples for the chronosequences of eucalypts and pine respectively, are shown in Figs. 5.1a and 5.1b. Different studies have demonstrated that these curves reflect the properties of the different organic compounds constituting the SOM, on the basis of their different thermal stabilities (Barros *et al.*, 2007).

In all soils, the DSC curves exhibit a prominent exothermic combustion peak in a narrow range of 325-339 ºC, which is attributed to the combustion of carbohydrates (Dell'Abate *et al.*, 2002; Lopez-Capel *et al.*, 2005), and named Exo 1 (Grisi *et al.*, 1998). The heights of these Exo 1 peaks varied greatly in the samples, following the changes in C content throughout afforestation.

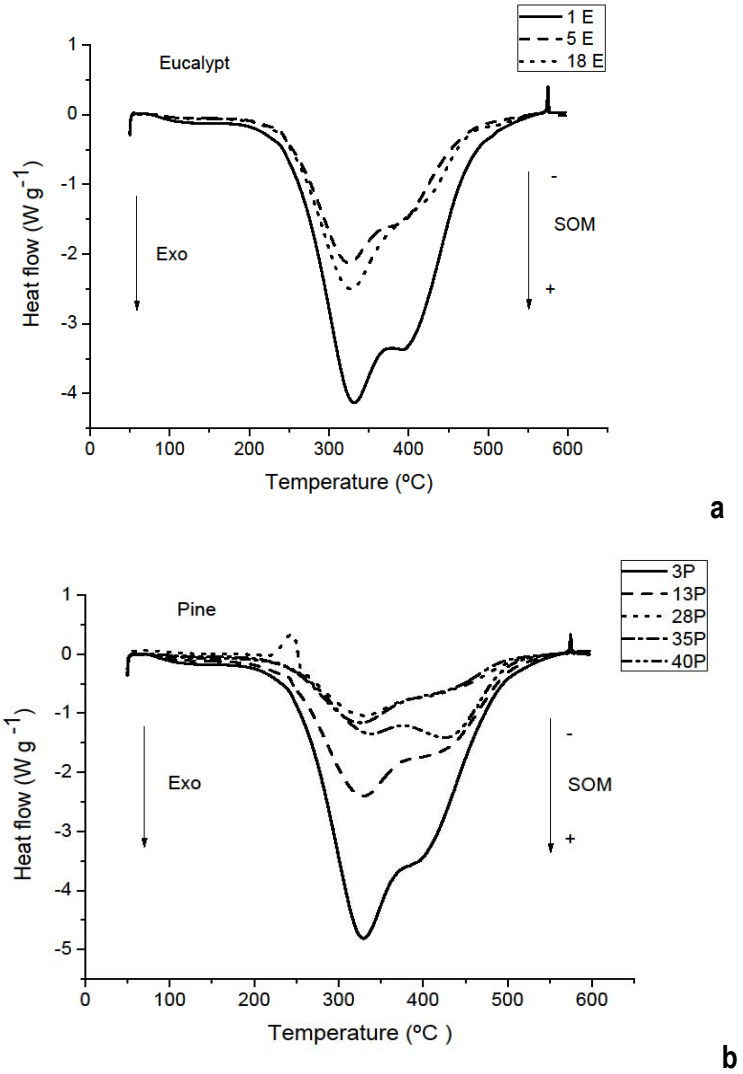

Figure 5.1. DSC curves for soil mineral samples (0-5 cm) from the *E. globulus* stands (Fig. 5.1a) and *P. radiata* stands (Fig. 5.1b). Different heights reflect different SOM contents. The name of the sample denotes the age of the stand and the tree species (e.g. 1E: one-year-old eucalyptus stand). Each curve represents the average of three replicates.

In accordance with the higher SOM contents, the samples of the youngest stands of each chronosequence (1E and 3P) exhibited the highest Exo 1 peaks (Figs. 5.1 and 5.2). In these samples, a secondary peak, named Exo 2, with a maximum at about 380 ºC and a tail until about 500 ºC, was also distinguished. In this peak the heat flow generated can be attributed to aliphatic C and fulvic acids (Cuypers *et al.*, 2002; Barros *et al.*, 2011), whereas the heat flow at temperatures higher than 400 ºC would reflect the presence of aromatic compounds in the SOM (Leinweber & Schulten, 1992; Leinweber & Schulten, 1999). Thus, according to this interpretation, the SOM in these samples, corresponding to the first years after afforestation, was characterized by large amounts of carbohydrates and carboxylic C, and lower amounts of aliphatic and aromatic compounds.

Table 5.3. Thermal properties of the soil samples. Heat of combustion (Q) of the soil, and combustion temperatures for Exo 1 and Exo 2 peaks in the DSC curves. Tª end is the temperature at which the combustion ended. The names of the samples denote the age of the stand and the tree species (e.g. 1E: one-year-old *E. globulus* stand). Data represent the average of 3 replicates. N=3 ± SD.

Tree Species	Samples	Q (kJ g^{-1})	Exo 1 T_1 (ºC)	Exo 2 T_2 (ºC)	Tª end (ºC)
E. globulus	1E	3.92±0.06	332 ±1	395±1	539±3
	5E	1.91±0.01	325 ±2	395±2	505±5
	18E	2.13±0.03	329 ±1		490±1
P. radiata	3P	4.45±0.09	330±1	398±2	539±2
	13P	2.54±0.03	330±1	417±5	520±1
	28P	1.08±	331±1	414±3	502±1
	35P	1.13±0.01	326±1	427±2	502±1
	40P	1.65±0.01	339±1	427±1	516±4

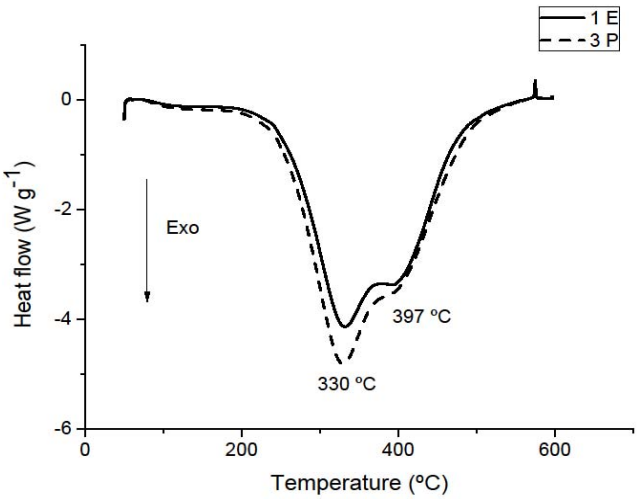

Figure 5.2. DSC curves for samples taken in the most recent afforested soils (1E and 3P are the samples taken from *E. globulus* and *P. radiata* plantations, 1 and 3 yr after afforestation, respectively). The combustion temperatures indicate the same SOM composition in both samples. Each curve represents the average of three replicates.

Characterization of SOM in the soils from the youngest stands was complemented by ^{13}C CP-MAS NMR analysis for the *P. radiata* samples (Fig. 5.3). The ^{13}C CP-MAS NMR spectrum of the sample 3P, revealed the presence of signal corresponding to saturated aliphatic chains, branched alkyl-C and CH_3O-C groups (0-60 ppm), carbohydrate C (55-110 ppm), aromatic groups (110-165 ppm) and carboxylic C (165-200 ppm). The most prevalent signals in the ^{13}C CP-MAS spectrum correspond to a broad band in the region 45–110 ppm. This region is typical of O-alkyl C groups and is generally attributed to polysaccharide material such as cellulose (Kögel-Knabner, 1997). In the aliphatic region, the spectrum shows two peaks, one at ~32 ppm assigned to acetyl C and methyl C in lipid, cutin, suberin or amino acids (Golchin *et al.*, 1996), and the other at 55 ppm,

which may reflect methoxyl and amino acid C (Cuypers *et al.*, 2002). Two peaks in the aromatic region at 130 and 150 ppm may be derived from lignin compounds and represent protonated, C substituted and O-substituted aromatic C respectively (Knicker & Lüdemann, 1995). Tannin and tannin-like structures may also contribute to the signal intensity in this region (Alexis *et al.*, 2010). The integration of the spectra of sample 3P in Fig. 5.3 yielded the following contributions to the NMR signal: 58% carbohydrates, in which cellulose contributes 22% to the carbohydrate composition; 5% of carboxylic groups, 17% of aliphatic groups and 11% of aromatic C.

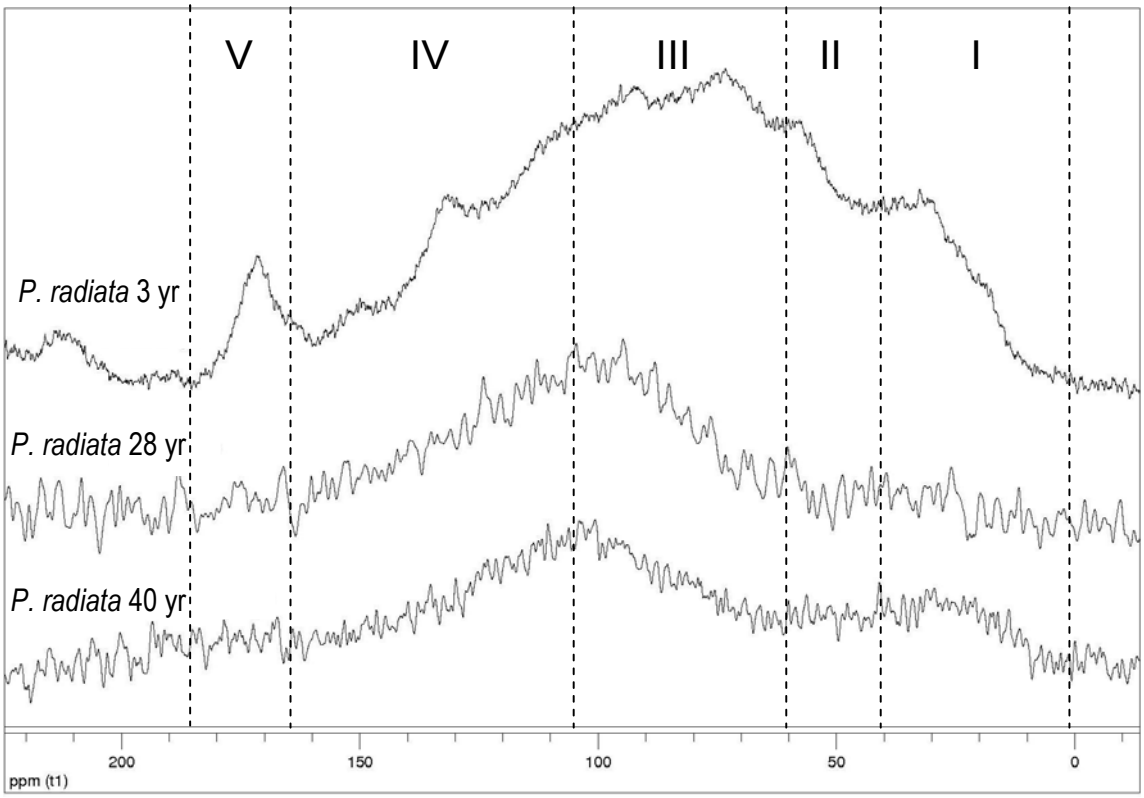

Figure 5.3. ^{13}C CP-MAS NMR spectra of selected soil samples (0-5 cm) from the *P. radiata* stands and relative integration areas: I (C in straight chain, branched and cyclic alkanes, II (C in branched aliphatics, amino acids and OCH_3 groups), III (C in carbohydrates and aliphatics containing C bound to OH), IV (aromatic C), and V (C in carboxyl, amide and ester groups).

For the soil samples in the stands at 1/3 of each rotation (5E and 13P), losses of 50% with respect to youngest stands were observed for the soil carbon concentration (Fig. 5.2). For both of these samples, the DSC curves show that the Exo 1 peak appears at the same temperature as in the youngest stands (1E, 3P), but at a much lower intensity. The temperature of the Exo 2 peak in the 5E sample also did not change with respect to that of the youngest site, but for the sample 13P it increased from 380 ºC to about 430-440 ºC, a temperature attributable to the combustion of aromatic C. The lower intensity of the Exo 2 in this latter DSC curve with respect to sample 3P indicates important depletions, not only in the more readily decomposable compounds, such as carbohydrate C and carboxylic C, but also in saturated aliphatic chains in the pine chronosequence.

In the *P. radiata* chronosequence, both samples at ages 28 and 35 years (28P and 35P) showed SOM depletions of about 75% relative to the youngest plantation. The DSC curves areas were extremely small, even in the ranges of temperatures of aromatic groups (combustion temperatures higher than 400 ºC). This finding is consistent with the ^{13}C CP-MAS NMR spectrum of sample 28P (Fig. 5.3), which reveals an important breakdown of carbohydrates (65-95 ppm), depletion of aliphatic groups (0-60 ppm), and loss of aromatic and phenolic groups (110-145 ppm and 145-165 ppm respectively), which are supposed to be highly recalcitrant to degradation. In addition, the low C/N ratios (close to 10) observed in these soils (Table 5.2) may reflect the loss of this type of compound.

Finally, in the soil samples collected at the end of the rotations (18E and 40P), which showed partial SOM gains, the DSC curves revealed increases in carbohydrates (higher Exo 1 peaks), especially in the soil under eucalyptus (Fig. 5.4). Interestingly, in sample 40P, the Exo 2 peak was higher than the Exo 1 peak, and also was found at higher temperature (427 ºC) than that of sample 18E (410 ºC). This suggests that at the end of the rotation more aromatic compounds were accumulated in the soil from the pine stands. The ^{13}C CP-MAS NMR spectrum of this sample showed two peaks (Fig. 5.3), one in the aliphatic band, representing 21% of the total area of the NMR spectrum, and a second one from the carbohydrate to the aromatic region representing 56% of the area of the spectrum.

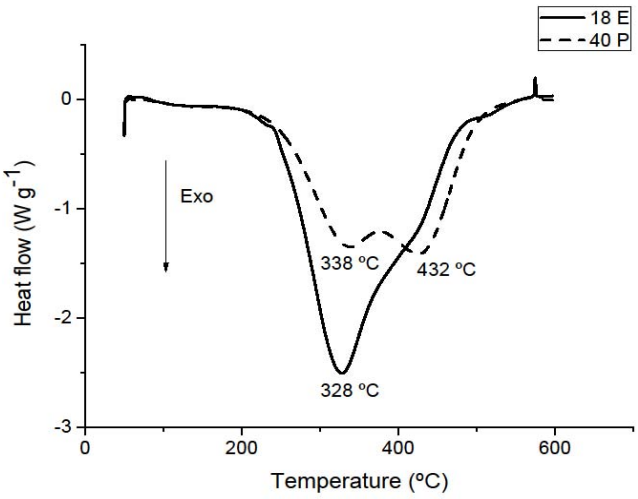

Figure 5.4. DSC curves for soil samples collected at the end of the rotation for each tree species (18E and 40P are the samples from *E. globulus* and *P. radiata* plantations, obtained 18 and 40 yr after afforestation, respectively). Differences in the SOM composition can be attributed to the tree species. Each curve represents the average of three replicates.

5.3.3. Measurement of basal metabolism and calorespirometric ratio by calorimetry

The heat rate (Φ) and the CO_2 rate (RCO_2) due to basal metabolism of the soil samples under the afforested pine and eucalypts stands throughout the measurement are shown in Figs. 5.5 and 5.6.

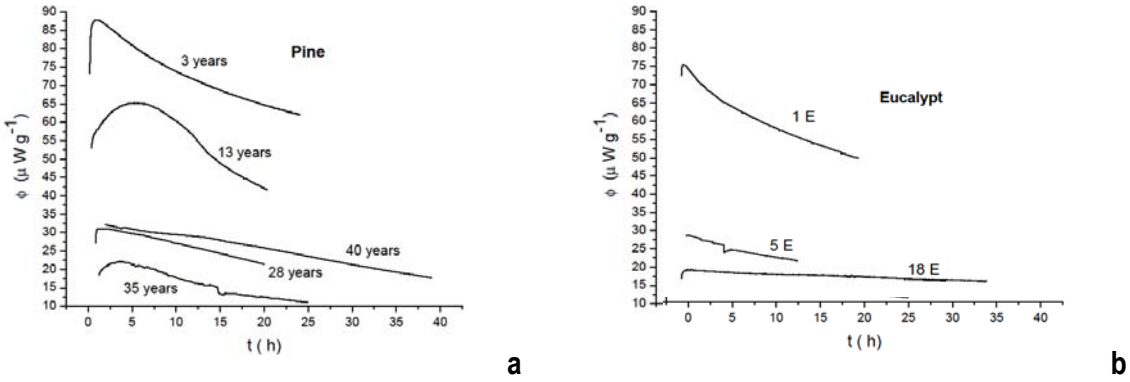

Figure 5.5. Power-time curves for the soil samples (0-5 cm) from the *P. radiata* stands (Fig. 5.5a) and from the *E. globulus* stands (Fig. 5.5 b). The names of the sample denote the age of the stand and the tree species (e.g. 1E: one-year-old *Eucalyptus* stand). Each curve represents the average of three replicates.

Most of the samples showed strong temporal decreases in the basal metabolism during the measurement of the heat rate (Fig. 5.5a and 5.5b) and of the CO_2 rate (in Figs. 5.6a and 5.6b). A decrease in metabolic rate has previously been observed for soil basal respiration measurements, and attributed to exhaustion of the more labile organic matter (Fang *et al.*, 2005).

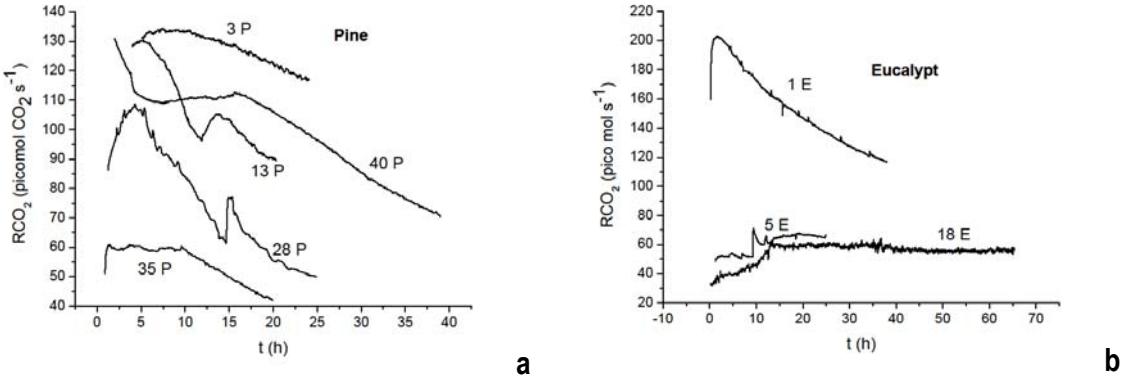

Figure 5.6. CO_2 production rate (RCO_2) of the samples from the *P. radiata* stands (Fig. 5.6a) and *E. globulus* stands (Fig. 5.6b) throughout measurement in the calorimeter. The names of the sample denote the age of the stand and the tree species (e.g. 1E: one-year-old eucalyptus stand). Each curve represents the average of three replicates.

The quotient between the heat rate and the CO_2 rate yields the calorespirometric ratio (Φ/RCO_2) of the soil basal metabolism (Fig. 5.7). This ratio reflects the efficiency of conversion of substrate into anabolic products, and provides information about the nature of the substrate carbon source (Edelstein *et al.*, 2001; Macfarlane *et al.*, 2002; Joyal *et al.*, 2005). The Φ/RCO_2 is directly related to the oxidation state of the substrate being metabolized and thus provides information

about the nature of that substrate. Degradation of carbohydrates yields Φ/RCO_2 values ranging between -200 and -455 kJ mol^{-1} CO_2, while Φ/RCO_2 values higher than -455 kJ mol^{-1} CO_2 occur when degradation of substrates that are more reduced than carbohydrates takes place (-570 kJ mol^{-1} CO_2 for proteins and -660 kJ mol^{-1} CO_2 for lipids, Hansen *et al.*, 2004).

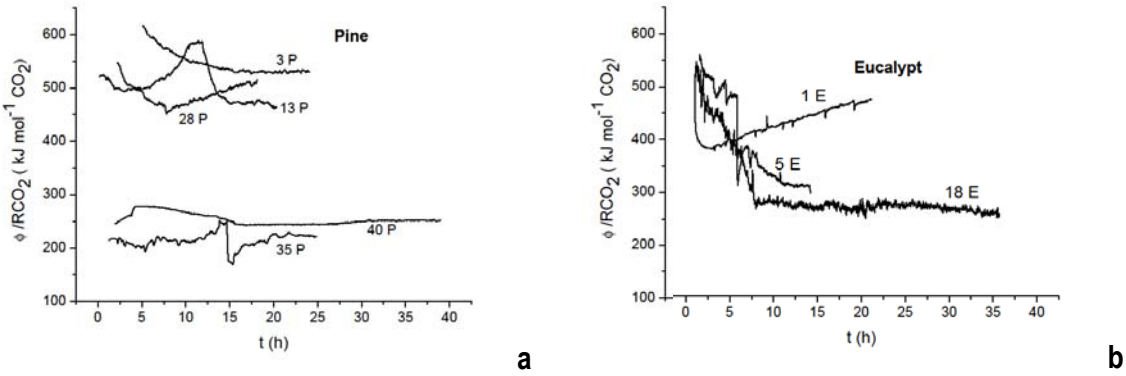

Figure 5.7. Ratio of metabolic heat rate to CO_2 rate (Φ/RCO_2) throughout the measurement in the calorimeter in the soil samples from the *P. radiata* stands (Fig. 5.7a) and the *E. globulus* stands (Fig. 5.7b). The names of the sample denote the age of the stand and the tree species (e.g. 1E: one-year-old eucalyptus stand). Each curve represents the average of three replicates.

The Φ/RCO_2 varied greatly in the samples studied, although most values then tend to stabilize over time (Fig. 5.7), indicating changes in soil microbial metabolism during the measurement. Such changes in response to soil disturbances probably also occur in nature. Thus, the soils under eucalypts (Fig. 5.7b) showed initial Φ/RCO_2 values of about -550 kJ mol^{-1} CO_2, indicating substrates that are more reduced than carbohydrates (Hansen *et al.*, 2004; Wadsö *et al.*, 2004). These samples showed important decreases in the Φ/RCO_2 until reaching a steady state. The Φ/RCO_2 values in the steady state of these samples were in the range given for carbohydrate respiration. The changes in Φ/RCO_2 in samples 3P and 13P (Fig. 5.7a) also showed more reduced values than for carbohydrates. The Φ/RCO_2 of the other samples from the pine stands were always within the range given for carbohydrate respiration.

5.3.4. Soil microbial activity in relation to the energy of the SOM

Results for the average tabulated values calculated for the metabolic rates (Φ, RCO_2) and for the calorespirometric ratios (Φ/RCO_2) are shown in Table 5.4. This table also includes the microbial biomass of the samples, the C_{mic} to C ratio and the metabolic quotient, qCO_2, all measured by calorimetry.

The important SOM losses that were found to occur after afforestation were accompanied by decreases in microbial biomass and basal metabolism (Φ, RCO_2), whereas the metabolic quotient qCO_2 showed the opposite trend. At the end of the rotation period, the subsequent increases in SOM gains led to slight recoveries of the values of these parameters.

Heat rate (Φ) and RCO_2 of the soil basal metabolism were positively correlated with the heat of combustion of the soil (Q), determined by DSC (R^2 = 0.94; R^2 = 0.81, respectively). Both indices were also correlated with SOC (R^2 = 0.77 and R^2 = 0.70, respectively) and the soil active microbial biomass (R^2 = 0.94 and R^2 = 0.68, respectively). The SOC was correlated with the soil microbial biomass (R^2 = 0.75).

Table 5.4. Data related to the soil microbial metabolism in the samples: the ratio between the biomass carbon and total soil C (C_{mic}-C), the heat flow rate of basal metabolism (Φ), the CO_2 production rate (RCO_2), the metabolic quotient (qCO_2), and the calorespirometric ratio (Φ/CO_2).

	Samples	C_{mic}-C(1)	Φ (μ W g^{-1})	RCO_2 (Picomol s^{-1})	qCO_2(2)	Φ/RCO_2 (kJ mol^{-1})
E. globulus	1E	5.0 ± 0.1	61±7	153±2	1.19 ± 0.04	434 ± 55
	5E	7.5 ± 0.5	30±2	61±4	0.67 ± 0.05	398 ± 30
	18E	3.0 ± 0.2	16±1	54±3	0.87 ± 0.07	310 ± 23
P. radiata	3P	6.2 ± 0.1	73±2	128±4	0.72 ± 0.09	546 ± 22
	13P	7.3 ± 0.3	38±1	109±2	0.99 ± 0.20	512 ± 15
	28P	7.4 ± 0.1	27±2	55±4	0.80 ± 0.09	491 ± 53
	35P	1.6 ± 0.1	16±5	76±22	4.75 ± 0.60	216 ± 95
	40P	2.7 ± 0.2	23±3	92±10	2.17 ± 0.58	252 ± 43

Units: (1) C_{mic}-C : mg Cmic g^{-1} C; (2) qCO_2 : μgCO_2-C μg^{-1}Cmic h^{-1} 10^{-2}

There was also a strong positive correlation between the CO_2 and Φ (R^2 = 0.84). The value of the slope was -481 kJ mol^{-1} CO_2, which is close to the Thornton´s constant (-455 kJ mol^{-1} CO_2) (Hansen *et al.*, 1997) and to that of -435 kJ mol^{-1} CO_2 calculated for soils by calorimetry and respirometry (Albers *et al.*, 1995). These results demonstrate the connection between the energy of the substrate and the microbial metabolism in soil, which is well described in the literature on the thermodynamics of microbial reactions (Cordier *et al.*, 1987; Sandler & Orbey, 1991).

Significant correlations between Φ/RCO_2 and microbial biomass (R^2 = 0.83) and C_{mic}-C (R^2 = 0.87) were found. These reflect that the nature of the substrates being degraded in soil (as measured as Φ/RCO_2) directly affects the C availability to microbial biomass. This ratio was also correlated with the efficiency of the microbial metabolism, qCO_2, (R^2 = -0.73), which shows that soil microorganisms need to develop metabolic adaptation mechanisms due to the changes in the nature of the SOM.

5.3.5. Changes in the Φ/RCO_2, qCO_2 and C_{mic}-C in the afforested soils

The temporal changes in the above indices over the time since afforestation (measured by calorimetry), are shown in Fig. 5.8 and Table 5.4. The Φ/RCO_2 values in samples from the most recently afforested stands (1E, 3P) were higher than -455 kJ mol^{-1} CO_2, indicating that the aliphatic and aromatic SOM fractions are being metabolized. The Φ/RCO_2 values in samples from pine stands 13P and 28P were also higher than the values for carbohydrates. The data are consistent

with the changes in the DSC curves and NMR spectra of those samples, which indicate degradation of aromatic C throughout the first years after afforestation (Figs. 5.1 and 5.3).

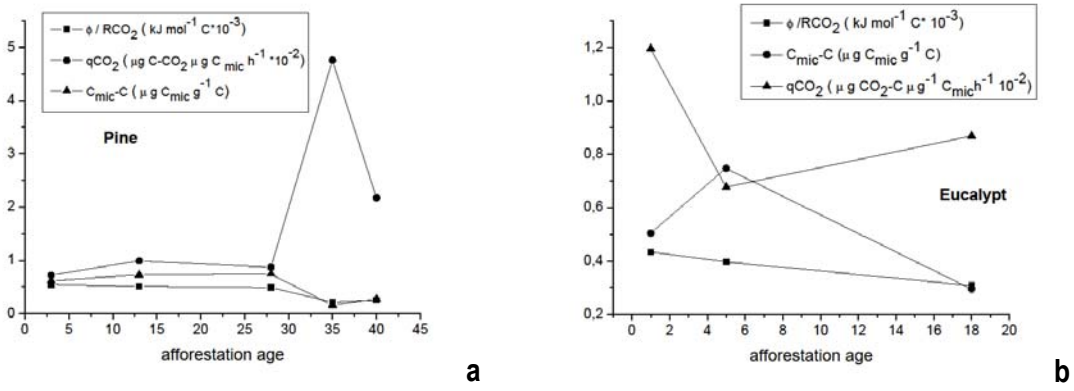

Figure 5.8. Changes in the ratio of metabolic heat rate to CO_2 rate (Φ/RCO_2), metabolic quotient (qCO_2) and ratio soil microbial biomass to SOC (C_{mic}-C) throughout the two chronosequences of *P. radiate* (a) and *E. globulus* (b). Each value represents the average of three replicates.

On the contrary, the Φ/RCO_2 values in the oldest sites (18E, 35P and 40P) were within the range given for carbohydrates (-200 and -455 kJ mol^{-1} CO_2). Coinciding with this change, these samples also showed higher qCO_2 and lower C_{mic}-C ratio than those from most recent afforested stands, which suggests a change in the metabolism pattern of the microorganisms relative to the efficiency of C use.

5.4. Discussion

5.4.1. Changes in the SOM in the afforested soils, as determined by calorimetry and thermal analysis

The data in this study revealed important losses of SOC in the mineral soils of the recently afforested stands. Depletions in SOC during the 30 years after forest establishment have been reported for different conditions. The effect has been attributed to increased mineralization of SOM, which is not compensated by any input of C from the fresh litter. The intensity of this loss is determined by the different factors affecting litter input and SOM decomposition, such as prior use, climate or the type of litter (Paul *et al.*, 2002; Vesterdal *et al.*, 2002; Cerli *et al.*, 2008; Foote & Grogan, 2010). The loss of SOM recorded in this humid temperate area is comparable to that observed in other afforestations carried out in similar forest systems in warmer climates (Zinn *et al.*, 2002).

In comparison with the pine stands, the eucalyptus stands lost less SOM and the period of SOM loss was shorter. The same pattern has been observed in different chronosequences in the

region (Chapter II) and was possibly due to the different dynamics of the litter accumulation and understory development in these types of plantations throughout the rotation. In pine plantations, canopy closure occurs within a short period, which greatly reduces the solar radiation from the third year onwards. This reduces the development of understory vegetation and favours litter accumulation. On the contrary, in the *E. globulus* plantations, the higher solar surface radiation promotes the development of plants, which, at the beginning of the rotation, consist of grasses (González-Hernández *et al.*, 1998). The litter accretion in the eucalypt stands was much slower, possibly because the higher decomposition rate of eucalypt litter, which has been described in the region (Alvarez *et al.*, 2008) and elsewhere (Paul & Polglase, 2004; Lemma *et al.*, 2007)

Thus, it is possible that the presence of grass, along with the higher litter decomposition rates resulted in greater C inputs in the eucalypt plantations. The litter derived from the grass vegetation (root biomass and root exudates), which is dominated by carbohydrates that decompose relatively easily, would explain the lower loss of SOM and the higher amounts of carbohydrates in these afforested soils. This latter was revealed by the shape and size of the DSC at the end of the rotation, which exhibited a prominent peak at 328 ºC. In accordance with this, Φ/RCO_2 values lower than -400 kJ mol^{-1} CO_2 were observed from the first stages of the stand development, suggesting respiration of carbohydrates as the main source of C for microorganisms.

Unlike in the eucalyptus stands, understory vegetation in the pine stands is very scarce and litter accumulates rapidly. This implies lower C inputs during the first years after afforestation. The DSC and NMR techniques revealed important losses of easily degradable compounds, such as carbohydrates, carbonyl/carboxyl groups, and even aliphatic and aromatic components, after afforestation. In the oldest stands of the pine chronosequence, the DSC and NMR technique revealed important losses of aromatic components, which are supposed to be more resistant to microbial degradation (Baldock *et al.*, 2004). In agreement with these trends, the changes in Φ/RCO_2 values (higher than -400 kJ mol^{-1} CO_2) during the first years after afforestation indicate microbial degradation of substrates that are more reduced than carbohydrates. The depletion of this type of compound at the end of the rotation period explains the low Φ/RCO_2 (lower than -300 kJ mol^{-1} CO_2), which indicates that carbohydrates were the main C source for microorganisms, possibly those that are supplied by plant decomposition.

These pine stands contain abundant litter, which contains recalcitrant compounds such as tannins and lipids. The production of resins, waxes, cutin and suberin in this type of litter (Chefetz *et al.*, 2002) explains the presence of more recalcitrant compounds at the end of the rotation (sample 40P). The presence of this type of compound was revealed by a prominent Exo 2 peak in the DSC curve (432 ºC) for the soil samples at the end of the rotation, as well as by an increase in compounds in the range 0-45 ppm in the NMR spectrum. Similar characteristics of SOM composition attributable to the type of litter developed on the mineral soil have been reported by Ostertag *et al*. (2008).

These results suggest that under these environmental conditions (humid temperate climate), the process of SOM depletion following afforestation does not only affect the most readily

decomposable compounds, but also the more stable pools. Although most studies on SOM dynamics in relation to afforestation report losses of easily decomposable cellulose and hemicellulose, and preservation of recalcitrant organic compounds (Cerli *et al.*, 2008), recent studies have demonstrated that degradation of the more recalcitrant SOM occurs in surface horizons, as found here (Fierer *et al.*, 2003; Goberna *et al.*, 2006; von Lützow *et al.*, 2006; Dorodnikov *et al.*, 2007). The soils of the present study contain low percentage of clay, whose fraction is dominated by minerals of low surface reactivity. Therefore, the capacity to stabilize C in mineral-associated forms and in fine pores can be limited. Nevertheless, the presence of Al and Fe should have contributed to the stability of soil organic matter.

5.4.2. Monitoring the SOM composition by DSC and RMN

The data in this study show that both DSC and NMR appear to be sensitive methods of monitoring the changes in SOM composition associated with afforestation. In fact, both methods are becoming popular for studying the structure and composition of SOM (Lopez-Capel *et al.*, 2005; Marín-Spiotta *et al.*, 2008). The changes in SOC throughout the rotation in the afforested stands resulted in changes in the shapes and sizes of the DSC curve, as well as in the temperatures of the combustion peaks.

The existence of carbohydrates and aliphatic C in the soil was detected by both DSC and ^{13}C CP-MAS NMR techniques. In the DSC curves, these compounds yielded combustion peaks at 325-339 ºC and 380 ºC, respectively. The NMR spectra yielded peaks in the 55-110 and 0-60 ppm regions respectively.

However, the identification of the aromatic fraction is not as clear. In the soil samples with high SOM content (e.g. sample 3P in Fig. 5.3), these substances appear in the 110-165 ppm region of the ^{13}C CP-MAS NMR spectra. In the DSC curve the presence of these compounds should appear as a combustion peak at temperatures higher than 400 ºC (Plante *et al.*, 2009). However, it appears that the high aliphatic contents of these samples may have obscured this peak, as the peak combustion of aliphatic and aromatic compounds occurs at similar temperatures. Thus, the existence of both types of compounds, aliphatic and aromatic C, generated an Exo 2 peak below 400 ºC,. In that case, the DSC curve area is broader, with a final combustion temperature of about 530 ºC. The samples without the aliphatic fraction in the NMR spectra have a final combustion temperature of about 502 ºC

On the other hand, soils with low C content yielded spectra with a very broad peak from the carbohydrate to the aromatic region (100-165 ppm, Fig. 5.3). It is well known that for low C contents (5 g kg^{-1}), it becomes difficult to acquire a ^{13}C CP-MAS NMR spectrum with an acceptable signal-to-noise ratio (Kögel-Knabner, 1997). In these cases DSC appears to be more sensitive than ^{13}C CP-MAS at differentiating the carbohydrate from the aromatic C (see DSC curves of 28P, 35P and 40P in Figs. 5.2 and 5.4).

Such problems related to the sensitivity of these two methods have been reported recently (Barros *et al.*, 2011). It is clear that the resolution of both ^{13}C CP-MAS NMR and DSC depends not only on the nature and quantity of the SOM, but also on the relation and proportions between the quantities of the different SOM compounds. Thus, DSC does not provide an accurate relation between the thermal properties and the nature of the SOM in samples with high percentages of C and a high diversity of SOM, although it distinguishes the carbohydrate from the aromatic fraction in samples with low C content and poor diversity of SOM. This technique also has the advantage of providing reproducible results through simple and rapid experimental phases, avoiding the use of reactants and handling of the soil.

According to these observations, application of both NMR and DSC provides more accurate information about the nature of the SOM, because they complement each other when applied to soil samples with a wide range of C contents.

5.4.3. Relating soil organic compounds to the pattern of soil microbial metabolism: the application of Φ/RCO_2

Φ/RCO_2 and SOM degradation

Calorimetry may be a valuable technique for understanding the relationship between microbial activity and SOM composition. Values of soil respiration in terms of the CO_2 rate (RCO_2), measured by the method proposed here are consistent with the values obtained by other methodologies that also use NaOH (Ge *et al.*, 2009; Marinari *et al.*, 2010). However, one of the main advantages of the calorimetry technique in the study of soil microbiology is that it enables continuous monitoring of the basal metabolism, expressed as both heat rate (Φ) and soil respiration (RCO_2).

In addition, the ratio of these two parameters, Φ/RCO_2, provides useful information about microbial metabolism, with respect the type of compounds in the SOM. The observed changes in Φ/RCO_2 in the samples were consistent with the changes in SOM in the DSC curves and NMR spectra. This enables better interpretation of the changes in the DSC curves over the time since afforestation, and a better understanding of the relation between the soil thermal properties and SOM degradability. There is still a lack of information in that sense which limits the application of DSC in these studies; recent studies have recommended further investigation of this aspect (Plante *et al.*, 2009; Duguy & Rovira, 2010).

The measurement of the soil microbial metabolism by this method also provides more insight into the reactions taking place in soil, since it can directly detect the degradation of aromatic and aliphatic compounds, which in this case enabled interpretation of the observed changes in the DSC curves. The biochemical reactions involved in the oxidation of those macromolecules cannot be monitored by the exclusive measurement of the CO_2 rate, because the biochemical pathways used to degrade aliphatic C chains do not release CO_2 during the first phases of the oxidation

process, and involve carboxylation reactions, which in some cases release heat but consume CO_2. This may also explain why the correlation between microbial biomass and RCO_2 (R^2 = 0.68) was weaker than that between microbial biomass and Φ (R^2 = 0.94), and also the low correlation between the heat and CO_2 rate reported by other authors (Critter *et al.*, 2004a).

The present study shows that the changes in SOM trend in the mineral soil in the afforested stands were similar to those observed for microbial biomass and activity. Such changes in the microbial metabolic patterns are consistent with those observed in other afforested soils (Merino *et al.*, 2004; Mao & Zeng, 2010) and are caused not only by the amount of SOC, but also by the changes in SOM composition. The Φ/RCO_2 data shown here provide more comprehensive information about the observed trend through the same experimental measurement, and indicate that degradation of substrates that are more reduced than carbohydrates takes place in soils with high SOM content and diversity, which have higher biomass and higher degradation rates than soils with poor SOM diversity, as previously described (Hopkinson *et al.*, 1998). This demonstrates that the degree of reduction of SOM affects the microbial activity, but does not mean that aliphatic and aromatic SOM (more reduced than carbohydrates) can not be degraded. On the contrary, Φ/RCO_2 values fell to between -200 and-250 kJ mol^{-1} CO_2 in samples containing low amounts of SOM, reflecting a change in the microbial metabolism to degradation of single carbohydrates as the main C source. This only occurred after degradation of the aliphatic and aromatic SOM fractions. When applied to the soil chronosequences under study, the values obtained suggest that at the end of the rotation, the only source of C in the soil is the fresh carbohydrates derived from litter decomposition, and that the microbial biomass is adapted at that point to that type of substrate. This promotes the accumulation of new aliphatic/aromatic fractions, as shown by the results of the DSC and NMR analyses of the soils at the end of the rotation. This new source of SOM is probably directly derived from the surface layer and the roots, which are directly associated with the forest species used in the afforestation.

Φ/RCO_2 provides general information about microbial metabolism

The results of this study show that Φ/RCO_2 is closely associated with the metabolic efficiency of the soil microbial biomass (measured as qCO_2), and with the availability of SOM to soil microorganisms (expressed as C_{mic}-C). This also enables a better understanding of the changes in qCO_2 and SOM.

The qCO_2 is the amount of CO_2 respired per unit of microbial biomass, and is considered a measure of microbial efficiency (Thiet *et al.*, 2006). All the samples in this paper degraded the SOM through quite stable qCO_2 values when the Φ/RCO_2 ranged from -546 to -310 kJ mol^{-1} CO_2. The increased qCO_2 observed in the soils at the end of the rotation, which degrade single carbohydrates from the litter through Φ/RCO_2 values below -310 kJ $mol^{-1}CO_2$, may indicate adaptation of microbial metabolism in response to the low SOM quantity and diversity, or the existence of nutritional stress caused by the depletion of nutrients and/or by less degradable OM (Trumbore, 2000). The nutritional “stress” may also explain the decrease in the C_{mic}-C ratio

observed in samples 18E, 35P and 40P. The decay in the microbial biomass in these samples is also a factor that leads to higher qCO_2 values (Wardle & Ghani, 1995).

Thus, degradation of substrates that are more reduced than carbohydrates takes place through an efficient metabolism and the loss of quantity and diversity of the SOM causes metabolic changes that yield a less efficient metabolism in the samples at the end of the rotation.

In conclusion, the calorespirometric ratio, along with other biological parameters, such as qCO_2 and C_{mic}-C, may be a useful tool for studying the patterns of soil microbial metabolism. This parameter works as a complex and sensitive index that provides information about changes of metabolism in response to the nature and red-ox state of the substrate and to the availability of the SOM to microbial attack, yielding new insight about the biological mechanisms involved in the process.

5.5. Conclusions

Thermal and calorimetry techniques proved to be valuable tools for determining the main changes brought about by afforestation, in relation to the nature of the SOM and microbial metabolism. DSC and NMN provide useful information about the composition of the SOM because they complement each other when applied to soil samples with wide ranges of C contents. The combination of both techniques also enables analysis of larger numbers of samples, when required.

The relationship between SOM composition and microbial metabolism was successfully determined by isothermal calorimetry, by means of the calorespirometric ratio, along with other biological parameters obtained by this technique, such as qCO_2 and C_{mic}/SOC. The calorespirometric ratio was found to be a sensitive index that provided information about changes in metabolism in response to the nature and redox state of the substrate and to the availability of the SOM to microbial attack.

The proposed methodology distinguished differences in the SOM dynamics and microbial metabolism attributable to the tree species, through different understorey vegetation and litter accumulation composition. The humid temperate climate, and perhaps the lack of stabilization in mineral-associated forms and in fine pores, favoured large losses of SOM after afforestation, which even affected highly recalcitrant aromatic compounds. The changes in SOM composition led to alterations in the pattern of microbial metabolism, the efficiency of which was particularly affected by the redox state and energy of the substrates.

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Chapter VI

Carbon balance for different management practices for fast growing tree species planted on former pasture land in southern Europe

6. Carbon balance for different management practices for fast growing tree species planted on former pasture land in southern Europe

Abstract

Although it is known that forestry mitigates carbon emissions to some degree, it is not known to what extent changes in forest management regimes affect the carbon cycle. In a climate-change scenario, forest management schemes must be optimized to maximize product supply and minimize environmental impacts. It is difficult to predict the mitigating effects of different silvicultural regimes because of differences in the growth characteristics of each species, destination of products, and industrial efficiencies. The objective of the present study was to evaluate the effects of different management regimes for fast growing species in southern temperate Europe in relation to mitigating climate change.

A comprehensive study was carried out considering the C sink effect in biomass, soil and wood products, the substitutive effect of bioenergy, and particular conditions of the forest industry in southern Europe. The mechanistic CO_2Fix model was parameterized for three species used in fast growing plantations in southern Europe: *Eucalyptus globulus*, *Eucalyptus nitens* and *Pinus radiata*. Data from 140 plots covering the complete age range observed for each species were used to calculate changes in C stocks in aboveground biomass and organic and mineral soil, and to validate the parameterized model for these conditions. Additional information about the efficiency of forest industry processes in the region was also considered.

A strong bias in soil organic carbon estimation was observed, and attributed to overestimations in the decomposition rates of soil compartments. Slight bias was also observed in the carbon biomass estimation when forest specific yield models were used to simulate afforestation over former pasture land. As regards the model sensitivity, the Yasso model was strongly robust to turnover of leaves, roots and branches. The chip-wood production alternative yielded higher carbon stock in biomass and products, as well as in bioenergy substitution effect, than the sawn-wood production alternative. Nevertheless, the sawn-wood alternative was the most effective as regards the C stock in the soil. Site index had an important effect for all species, alternatives and compartments, and mitigating effects increased with site index. Harvesting of clearcutting and thinning slash for bioenergy use led to a slight decrease in the soil carbon equilibrium, but significantly increased the mitigation effect through bionergy use.

Keywords: *C sequestration, tree biomass, soil organic matter*, *CO_2Fix*

6.1. Introduction

The United Nations Framework Convention on Climate Change (UNFCCC) has recognised the importance of forestry plantations for mitigating greenhouse gases, as well as the need to monitor, preserve and enhance terrestrial carbon stocks (Updegraff *et al.*, 2004). Forest management in Europe generates considerable reductions in carbon emissions (Groen *et al.*, 2006) and Atlantic and central-mid European mountain sites have the largest carbon sequestration potential in Europe (Nabuurs & Schelhaas, 2002).

The Kyoto Protocol (UNFCCC, 1997) recognizes two main alternatives for emissions compensation in the Land Use, Land Use Change and Forestry (LULUCF) sector for Annex I countries, depending on whether actions took place in their own territory (Art. 3.3, 3.4 and 6) or in other non-Annex I countries (Art. 12). For actions inside their own borders, the Kyoto Protocol (KP) allows countries included in Annex I the following activities for compliance with the KP (UNFCCC, 1997; UNFCCC, 2001): compensation of greenhouse gas (GHGs) emissions through removal from direct human-induced land-use change and forestry activities, limited to afforestation, reforestation and deforestation since 1990 (Art. 3.3), and forest management activities (Art. 3.4).

The mitigation effects of forests can be achieved by maintaining or increasing the C stocks in the existing pools (biomass, soil and dead wood) or potential pools (wood products and bioenergy substitution). Options for enhancement and maintenance of carbon sequestration are reducing deforestation, expanding forest areas, increasing the carbon stock in existing forests (including soils), increasing the use and lifespan of wood products, and using wood products as biofuels for substituting fossil fuels (Kauppi *et al.*, 2001; Nabuurs & Schelhaas, 2002).

In this sense, large areas of agricultural land have been transformed to forest plantations in Spain since the implementation of EEC directive 2080/92 (EU 1992), which laid down a grant scheme for promoting reconversion of agricultural land to forest land. Although the effects on the C stock change in the system have been already studied (Chapter II), the effects of forest management on the C stock in this particular system are still not clear. Appropriate management of a forest system should guarantee maintenance of or an increase in C balance, independently of the management objectives, which sometimes focus on enhancement of carbon in forest biomass, although this has an impact on soils and wood products (Karjalainen, 1996). Proper management may be a crucial factor regarding soil, which can act as a source of greenhouse gases (CO_2 CH_4 and N_2O) or a sink (CO_2 and CH_4), depending on land use and land management (Lal, 1999).

The chain of forest activities related to afforestation covers many different silvicultural practices, which aim to produce specific products used by the forest industry, or for bioenergy purposes. Forestry in Atlantic south-western Europe is dominated by the use of fast growing species, which can be either introduced species (*Eucalyptus globulus* (Labill), *Eucalyptus nitens* (Dean & Maiden) Maiden, and *Pinus radiata* (D.Don) or native species (*Pinus pinaster*). Moreover, the forest industry relies strongly on timber transformation, and any study of the mitigating effects

of forest plantations should consider the specific transformation processes. There is a long history of forestry in northern Spain, which currently covers the range of primary transformation activities, to produce wood pulp, chipboard, sawn wood and plywood. These industries are also characterised by a high level of integration, whereby the industrial residues produced by one line of transformation are usually used by other industries with lower requirements for raw material. The use of each lot of wood depends upon the timber assortment and species. Production of bleached eucalypt pulp (BEP) is important as regards *E. globulus* and, to a lesser extent, *E. nitens*. There is no diameter threshold limit for pulp, but bark separation becomes more difficult when the small-end diameter is less than 7 cm over bark. The board industry (MDF, HDF or particle board) acts as a subsidiary of the pulp industry, consuming many different species of wood of small diameter.

The sawn wood industry mainly consumes *P. radiata* timber, with increasing interest in both *E. globulus* and *E. nitens* (Nutto & Touza Vázquez, 2004). The size limitation for this industry is 15 cm in small end for pines, and although this has not been defined for either *Eucalyptus* species, a threshold limit of 30 cm could be considered. Waste and sawdust from sawing are used by the board industry. The plywood industry requires the largest small-end diameters, and debarking is required.

An important share of the energy demands of wood-based industries are met by combusting the residual biomass generated during timber processing (mainly tree bark and sawdust). However, harvesting of thinning slash is increasingly implemented in management schemes. Although the maximum proportion of extracted harvest slash is 50% with the current machinery available (Tolosana, per. com.), this practice has some nutritional implications (Merino *et al.*, 2005; Balboa-Murias *et al.*, 2006), and the effects on global C mitigation are still under investigation.

Regarding C estimation, it is logistically impractical to sample the many sites, soil types, management regimes, landscapes and climatic regimes in a sufficiently detailed manner, to quantify the potential of SOC sequestration (Lemma *et al.*, 2007). Model development is therefore an essential tool for evaluating the mitigating effects of forest systems. In particular, soil models are valuable tools for understanding the mechanisms of SOC build-up (Liski *et al.*, 2005; Lemma *et al.*, 2007; Palosuo *et al.*, 2008). The development of accurate models for predicting forest C evolution can therefore provide valuable information for scientists and policy makers. Simulating forest growth with very detailed process-based models can only be executed at a limited number of sites because of the need for initialisation data and computing capacity (Karjalainen *et al.*, 2002), models must therefore be as simple as possible. In order to upscale to higher land levels (regional or national), models should represent the average growth conditions for the species considered and the selected climate zone (Karjalainen *et al.*, 2002), which is difficult with very simple models.

The objectives of this study were: (i) to parameterize the CO_2Fix model for plantations of *E. globulus*, *E. nitens* and *P. radiata* established over former agricultural land, (ii) to validate the results with a network of plots in which biomass and soil carbon density are measured directly, (iii) to evaluate the model sensitivity to several key parameters, and (iv) to establish the baseline of the

standard management alternatives for each species and to evaluate the effect of silviculture on the mitigating effect of these plantations.

6.2. Materials and methods

6.2.1. Site description and model used

The study was carried out in the temperate-climate zone of northern Spain. The average annual temperature is between 11.5 and 13.3ºC, precipitation is between 1213 and 1488 mm, with 411-808 mm distributed in the growing season (Table 6.1). The soil humidity and temperature regimes are Udic (mean period with partial drought, 1 month) and Mesic (mean frost-free period, 10 months), respectively. The soils were developed from granitic rocks, schist and shale, and classified as Humic or Distric Cambisols and Alumi-humic Umbrisols (IUSS Working Group WRB, 2006). The soil has a loam or sandy loam texture and is well drained. Other site characteristics regarding soil organic carbon (SOC), litter accumulation, and species productivity are shown in Table 6.2.

Table 6.1. Average climate conditions (and standard deviation) considering the geographic distribution of each species in north-western Spain.

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Annual
PRECIPITATION													Total
E. globulus	184.6	156.8	123.4	134.1	106.1	66.9	47.7	52.5	90.1	158.4	171.9	195.1	1487.6
	(56.2)	(49.4)	(35.1)	(31.7)	(25.9)	(15.1)	(16.9)	(15.3)	(24.4)	(34.1)	(43.8)	(59.8)	(376.9)
E. nitens	177.1	154.4	120.3	126.2	101.4	58.6	41.6	41.4	87.5	153.8	169.6	202.4	1434.3
	(51.5)	(42.2)	(33.7)	(35.9)	(18.6)	(14.9)	(8.8)	(4.7)	(22.0)	(33.1)	(44.2)	(47.6)	(322.4)
P. radiata	145.4	129.9	99.5	99.8	96.1	56.1	32.9	39.3	78.5	133.9	135.0	166.8	1213.2
	(27.8)	(29.6)	(22.2)	(24.3)	(14.8)	(13.0)	(11.3)	(7.9)	(14.4)	(26.6)	(36.0)	(27.0)	(219.7)
TEMPERATURE													Aver.
E. globulus	8.8	9.2	10.6	11.2	13.6	16.1	18.3	18.8	17.4	14.5	11.3	9.8	13.3
	(1.4)	(1.4)	(1.2)	(1.2)	(0.9)	(0.8)	(0.6)	(0.6)	(0.7)	(1.1)	(1.3)	(1.5)	(1.0)
E. nitens	6.4	7.0	8.6	9.0	12.2	14.8	17.4	18.0	16.0	12.8	9.1	7.4	11.6
	(0.6)	(0.5)	(0.7)	(0.7)	(0.5)	(0.5)	(0.6)	(0.8)	(0.5)	(0.6)	(0.7)	(0.8)	(0.5)
P. radiata	6.2	7.0	8.3	9.5	12.2	15.2	17.5	17.8	16.0	12.7	8.9	6.9	11.5
	(1.2)	(1.0)	(1.1)	(0.9)	(0.7)	(0.7)	(0.7)	(0.7)	(0.7)	(0.9)	(1.2)	(1.3)	(0.8)

The CO_2Fix model was used (Mohren & Goldewijk, 1990; Mohren *et al.*, 1999). Version 3.1 of the model (Masera *et al.*, 2003; Schelhaas *et al.*, 2004) enables quantification of the C stocks and fluxes with the full carbon accounting approach (Nabuurs *et al.*, 2002). This model is divided into six modules: biomass, soil, products, bioenergy, financial and carbon accounting. Model

profitability for even and uneven-aged forest stands has already been demonstrated, but special parameterizations have also been carried out, i.e. for non-forest systems, coppice, and catastrophic events (Schelhaas *et al.*, 2004; Groen *et al.*, 2006). Multi-cohort systems can also be simulated with this model.

Table 6.2. Stand characteristics of plots used for model validation.

Variable	*E. globulus* (n=40)				*E. nitens* (n=40)				*P. radiata* (n=40)			
	Aver.	Max.	Min.	S.D.	Aver.	Max.	Min.	S.D.	Aver.	Max.	Min.	S.D.
Age (year)	12.2	23.0	1.0	5.7	9.7	18.0	2.0	4.2	15.8	40.0	2.0	9.2
Site Index (m)*	23.3	36.3	9.0	6.5	15.3	27.7	8.8	4.4	24.8	34.8	17.4	4.4
N (stem ha^{-1})	1108	1687	446	309	1061	1675	650	286	1504	3400	675	622
SOIL CARBON (Mg ha^{-1})												
SOC Pasture (0-30 cm)	82.3	163.2	26.3	35.1	74.7	147.5	39.9	28.5	67.4	92.2	30.9	13.6
SOC Forest (0-30 cm)	79.5	141.0	40.2	25.2	68.6	106.7	41.2	16.9	63.1	125.9	12.0	24.7
Litter	8.5	62.2	0.0	11.1	25.5	49.3	1.5	11.4	27.2	69.3	0.0	20.9
BIOMASS CARBON (Mg ha^{-1})												
Total Forest	124.0	425.3	0.1	96.2	82.0	192.3	3.1	48.5	125.2	445.0	1.1	105.1

*Reference ages for each species are: 10, 6 and 20 years for *E. globulus*, *E. nitens* and *P. radiata* respectively.

The biomass module drives C changes in the other compartments. Live biomass growth must be provided in the model for the following fractions: stem, branches, leaves and roots. The model considers tree senescence mortality, as well as reductions in the biomass pool due to logging. Soil module inputs came from biomass turnover and logging residues, and soil dynamics are managed by the Yasso model (Liski *et al.*, 2005). The latter model considers three litter compartments and five decomposition compartments, in which organic matter transfer from fresh to more humified compartments depends on the chemical characteristics and on temperature and water availability. The most recent version of the CO_2Fix model incorporates an energy substitution module, which calculates the C-equivalent greenhouse gas flow differences between biomass-generated energy and energy derived from alternative fossil fuel. For more details about version 3.1 of the CO_2Fix model, see Masera *et al.* (2003) and Schelhaas *et al.* (2004).

6.2.2. Biomass module parameterization

The biomass module was parameterized as a function of stand age by using the yield models developed in Chapter IV for *E. globulus* and *E. nitens*. Since there were two forest-stand specific models available for *P. radiata* (Castedo-Dorado *et al.*, 2007; 2009), the suitability of both were tested. These models enable estimation of stem wood yield at the stand level in terms of current annual increment over bark (CAI, m^3 ha^{-1} yr^{-1}). Regional site quality models were used to transform dominant height to age for each species: for the single stem rotation of *E. globulus* (Fernández López*,* 1982), for *E. nitens* (Pérez-Cruzado, 2009), and for *P. radiata* (Diéguez-Aranda *et al.,*

2005). No mortality is included in the corresponding tab of the CO_2Fix model, since was captured in the yield models.

The suitability of yield models for biomass prediction was guaranteed for *E. globulus* and *E. nitens*, as the plots used for result validation were also used in developing the growth models (Chapter IV), whereas two forest-specific models were used to construct *P. radiata* yield tables. Results obtained from the static (Castedo-Dorado *et al.*, 2009) and dynamic (Castedo-Dorado *et al.*, 2007) models available for *P. radiata* in the region were compared with the present results, with regional adjustments with 40 plots used for model validation, all established over former agricultural land. For this, equations for d_g prediction from H_0 and N [6.1], and for stand volume over bark estimation (V, m^3 ha^{-1}) [6.2] were adjusted for 40 *P. radiata* plots established over former pasture land. The site characteristics of these data are shown in Tables 6.1 and 6.2. Equations [6.1] and [6.2] were fitted simultaneously by the MODEL procedure of the SAS/ETS® system (SAS Institute Inc, 2004), and heteroscedasticity was corrected as below.

$$d_g = b_0 \cdot N^{b_1} \cdot H_0^{b_2} \qquad [6.1]$$

$$V = b_3 \cdot d_g^{b_4} \cdot H_0^{b_5} \cdot N^{b_6} \qquad [6.2]$$

Wood density over bark (ρ, kg m^{-3}) was determined considering the dry weight of wood and bark estimated from biomass equations at tree level (Brañas *et al.* (2000), Chapter III, and Merino *et al.* (2005) for *E. globulus*, *E. nitens* and *P. radiata* respectively), and stem volume over bark estimated from regional taper functions (Sánchez *et al.* (2004), Pérez-Cruzado (2009), and Castedo-Dorado *et al.* (2007) for *E. globulus*, *E. nitens* and *P. radiata* respectively), both estimated until 7cm over small end bark.

Since there were no local models for root biomass estimation for both *Eucalyptus* species, the O'Grady (2005) and the Resh *et al.* (2003) models were used for *E. globulus* and *E. nitens* respectively. The same methodology was used for root estimation in Ethiopian plantations for CO_2Fix parameterization (Lemma *et al.*, 2007), as no local root biomass equations were available. With the exception of leaves and roots, the other biomass fractions considered in the CO_2Fix model are not the same as those used in the tree-level biomass equations The average carbon contents (C, %) were determined for branches and stem considering the proportion of each fraction considered in the biomass equations. The proportion of each biomass fraction was calculated from biomass equations, and a weighted average was calculated for C, for each tree measured in all plots. The average values of C in each CO_2Fix biomass fraction were estimated from carbon concentrations published for each biomass fraction considered (Merino *et al.* (2005), Chapter IV and Balboa-Murias *et al.* (2006) for *E. globulus*, *E. nitens*, and *P. radiata* respectively). Average values for ρ and C for each species considered are shown in Table 6.3.

Table 6.3. CO_2Fix model parameters used in simulations.

Parameter	Units	*E. globulus*	*E. nitens*	*P. radiata*
BIOMASS MODULE				
Carbon Stem	(%)	44.8	49.5	51.0
Carbon Branches	(%)	45.5	50.2	52.0
Carbon Leaves	(%)	52.0	57.2	52.7
Carbon Roots	(%)	45.2	50.1	49.7
Wood density	(kg m^{-3})	569.9	439.5	374.5
SOIL MODULE				
Leaves turnover	(yr^{-1})	0.324	0.264	0.360
Branches turnover	(yr^{-1})	0.0303	0.021	0.03
Roots turnover	(yr^{-1})	0.047	0.078	0.073
PRODUCT MODULE (threshold limit)				
log-wood	(cm)	30	30	20
pulp-wood	(cm)	5	5	5
BIOENERGY MODULE				
Slash low heating value	(MJ kg^{-1})	16.8	16.9	16.5

Additional models were developed in this study for determination of allocation coefficients, thus models for stem biomass (W_s, Mg ha^{-1}) defined as wood plus bark in the stem until 7cm in small-end, branch biomass (W_b, Mg ha^{-1}), leaf biomass (W_l, Mg ha^{-1}) and root biomass (W_r, Mg ha^{-1}) were fitted for each species considered. The same database of plots as described in Chapter IV for *E. globulus* and *E. nitens* was used for stand-level model construction, and the plots measured in this study and the biomass equations at tree level developed by Merino *et al.* (2005) for *P. radiata*. The models took the form described in equations [6.3-6.6], and enabled measurement of the biomass allocation in each biomass compartment. Values obtained for each simulation and age were set to 1, to determine the allocation factor for each compartment relative to stem biomass.

$$Ws_k = b_{k.1} \cdot d_g^{\,b_{k.2}} \cdot H_0^{\,b_{k.3}} \cdot N^{b_{k.4}} \quad [6.3]$$

$$Wb_k = b_{k.5} \cdot d_g^{\,b_{k.6}} \cdot H_0^{\,b_{k.7}} \cdot N^{b_{k.8}} \quad [6.4]$$

$$Wl_k = b_{k.9} \cdot d_g^{\,b_{k.10}} \cdot H_0^{\,b_{k.11}} \cdot N^{b_{k.12}} \quad [6.5]$$

$$Wr_k = b_{k.13} \cdot d_g^{\,b_{k.14}} \cdot H_0^{\,b_{k.15}} \cdot N^{b_{k.16}} \quad [6.6]$$

The equations were fitted by use of the MODEL procedure of the SAS/ETS® system (SAS Institute Inc, 2004). Because of the existence of correlation between error components of the independent variables and dependent variables, the full information maximum likelihood method

(FIML) was applied in the fitting process. Equations for each species were fitted simultaneously by the seemingly unrelated regressions method (SUR) to guarantee the additivity of the system (Parresol, 2001). The MODEL procedure of SAS was applied to obtain the SUR estimates, considering the parameters obtained in a previous individual fitting as initializers. Lack of homogeneity in the variance was corrected as previously reported (Chapter III).

6.2.3. Soil module parameterization

Died organic matter supply to the soil is the key factor in soil organic matter evolution, and accurate estimation is required for realistic simulations. Since litterfall and root turnover were not evaluated in the field, leaf, branch and root turnover were determined indirectly.

Leaf litterfall is a crucial parameter for eco-physiological growth models, i.e. 3-PG (Landsberg & Waring, 1997), where an accurate value of this parameter enables matching the observed yield in permanent plots. For leaf turnover (*LT*), parameters fitted for the 3-PG model for each species was considered: Sands and Landsberg (2002) for *E. globulus*, Pérez-Cruzado *et al.* (2011) for *E. nitens*, and Rodríguez *et al.* (2002a) for *P. radiata*. The 3-PG model considers two different *LT* depending on tree age, but although the initial litterfall is significantly lower, it is time limited relative to the maximum value, which tends to be asymptotic and dominates along the rotation (Sands & Landsberg, 2002). The values of *LT* considered (Table 6.3) are consistent with the observed average leaf lifespan for each species, and with reported litterfall for *P. radiata* (Santalla *et al.*, 2011) and *E. globulus* (Alvarez *et al.*, 2008) in the region.

Branch turnover (*BT*) was estimated considering the changes in tree and crown dimensions over time. It was considered that all branches have fallen when the living crown basis (h_{cb}) increases in the stem between time t_1 and t_2 (Fig. 6.1). For the species in which dead branches in the stem (w_{db}) are significant, the proportional part of this biomass that is held in the stem at moment t_2 was considered, in other cases *A* must be set to 0. Equation [6.7] was used to estimate the *BT* considering time intervals of one year and using the yield tables reported in point 6.2.2. Average tree dimensions were estimated for each time interval, where $\bar{d}$ was estimated from d_g using the equations developed in Chapter IV for *E. globulus* and *E. nitens*, and by Castedo-Dorado *et al.* (2007) for *P. radiata*. Total height was estimated from generalized height-diameter relationships for each species, and living crown basis (h_{cb}) was estimated from the equations of Nutto and Touza (2004) for *E. globulus*, our own model for *E. nitens* (data not shown) and that of Crecente-Campo (2008) for *P. radiata*. For *BT* an average value was obtained from $\bar{d}$ = 10 cm to $\bar{d}$ = 50 cm, values for *BT* for each species are shown in Table 6.3.

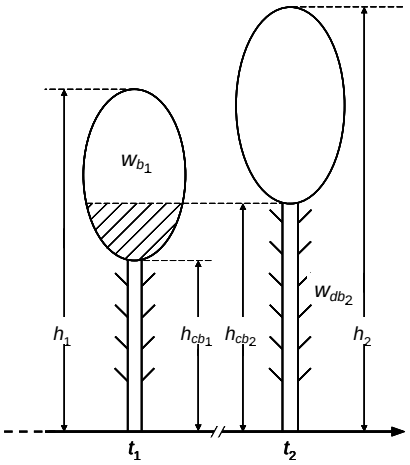

Figure 6.1. Determination of branch turnover from changes in individual tree dimensions over time: where: h is total height, h_{cb} is live crown basis, w_{db} is dead branch biomass in the stem, w_b is branch biomass in the crown, and t_1 and t_2 are two consecutive ages.

$$BT = \frac{\left(\frac{\left(h_{cb_2} - h_{cb_1}\right) \cdot \left[\left(\frac{w_{b_1}}{h_1 - h_{bc_1}} \right) - (A) \right]}{t_2 - t_1} \right)}{w_{b_1}} \quad \text{where} \quad A = \frac{w_{db_2}}{h_{bc_2}} \qquad [6.7]$$

Root turnover (*RT*) is not easy to determine, even when a specific experimental design is followed, and results vary greatly depending on the methodology used (Jourdan *et al.*, 2008). The turnover rate of total root systems depends on the turnover rate of each root size. It is known that turnover of fine roots is faster than that of thicker roots, and therefore the proportion of root sizes must be considered to obtain a global average value. Moreover, the proportion of different sized roots changes over time, with a higher proportion of fine roots in smaller tree sizes, and therefore higher global root turnover rates. *RT* was determined considering average root turnover rates of 0.915 yr^{-1} and 0.019 yr^{-1} for fine and coarse roots for both *Eucalyptus* species, and 0.786 yr^{-1} and 0.049 yr^{-1} for the *P. radiata* root system. These values were obtained from average values for *Eucalyptus* and *Pinus* species, both in a temperate region (Gill & Jackson, 2000). A weighted average was used for the turnover rates considering that the ratio of fine to coarse roots is the same as that of leaves plus twigs to the rest of the crown (Scarascia-Mugnozza *et al.*, 2000; Mund & Schulze, 2006). As the root biomass equations do not distinguish between stump and the other fractions, the proportion of the stem relative to the crown was considered to be the same as the stump relative to the other root fractions. The weighted average was calculated by assigning a turnover rate of 0 to the stump. *RT* values were obtained for the same tree dimensional classes as those considered for *BT*, and the arithmetic average was estimated for all of them. The *RT* values obtained for each species are shown in Table 6.3.

Detailed climatic data were compiled for each plot measured, and was corrected considering the elevation of each plot relative to the nearest climate measuring station. Average monthly temperature was corrected by a reduction of 0.5º C and an 8% increase in average monthly precipitation for each 100 m.a.s.l. increment in altitude relative to the climate monitoring station (Aguiló *et al.*, 1995). The July and August precipitation values were not corrected because the precipitation in these months is mainly of convective origin. Climatic data were obtained from the Galician network of climate monitoring stations of the Regional Natural Resources Administration (MeteoGalicia, 2009). Average values, representative of each species considered, were estimated for final simulations (Table 6.1). However, plot specific values were considered for parameterization validation.

6.2.4. Product and bioenergy module parameterization

For product module parameterization, the average harvested tree size was considered in each logging, and local taper functions (see point 6.2.2) were used for product classification for each destination. The threshold limits in the small-end for each destination (log-wood, pulp-wood and slash) are shown in Table 6.3. Logs and branches with diameter in the big-end up to 7 cm, plus foliage, were considered as slash.

Raw material allocation in each production line was carried out according to the real characteristics of the timber market in northern Spain (Toval, per. com.). The raw allocation coefficients considered two alternative silviculture regimes for each species: A, mainly aimed at the chip-wood for pulp or board industry, and B, mainly aimed at producing high quality sawn-wood, are shown in Table 6.4. No mill site dump was considered, because all industrial process losses are used for bioenergy, although locally generated biomass combustion ash contains some charcoal (Solla-Gullón *et al.*, 2008; Pérez-Cruzado *et al.*, 2011a; Santalla *et al.*, 2011), the final destination of which may be a mill site dump, or for use as a forest fertilizer or in brick fabrication.

Table 6.4. Product module parameterization for each species considered

RAW MATERIAL ALLOCATION		Sawn-wood		Boards		Paper		Firewood	
TO PRODUCTION LINES		A	B	A	B	A	B	A	B
E .globulus	Log-wood	0	0.4	0.2	0	0.7	0.5	0.1	0.1
	Pulp-wood			0.3	0.1	0.6	0.8	0.1	0.1
E. nitens	Log-wood	0	0.4	0.7	0.5	0.2	0	0.1	0.1
	Pulp-wood			0.6	0.8	0.3	0.1	0.1	0.1
P. radiata	Log-wood	0.3	0.7	0.6	0.2	0	0	0.1	0.1
	Pulp-wood			0.9	0.9	0	0	0.1	0.1
PROCESS LOSS REALLOCATION		Sawn-wood		Boards		Paper		Firewood	
(all species)	Sawn-wood			0.4		0		0.05	
	Chipboard					0		0.15	
	Paper							0.15	

Process loss reallocations to other production lines for all species are shown in Table 6.4. These were parameterized considering the real intrinsic characteristics of forest product market in northern Spain (Toval, per. com.). Product allocation factors among commodities and their characteristic lifespan, and product destination at the end of its useful life are shown in Table 6.5, and parameters of product destination at the end of life was considered as default for low processing and recycling efficiency (Schelhaas *et al.*, 2004). The lifespan of final products considered was 20 yr for long term products, 10 yr for medium term products and 1 yr for short term products.

Table 6.5. Product allocation to commodities and end-of-life destination (as a fraction of one).

PRODUCT ALLOCATION	Long term	Medium term	Short term
Sawn-wood	0.5	0.3	0.2
Boards	0.3	0.5	0.2
Paper	0.01	0.1	0.89
PRODUCT LIFESPAN (years)	20	10	1

Although product allocation to long term commodities (Table 6.5) was higher than considered as default in the low processing and recycling efficiency default parameterization, assumptions are still conservative, since the lifespan was significantly shorter than that considered in other studies (Row & Phelps, 1990; Karjalainen *et al.*, 1994; Karjalainen, 1996; Nabuurs & Sikkema, 2001; Karjalainen *et al.*, 2002). Default bioenergy parameters were considered, except for the slash fuelwood heating value, for which low heating values were local data reported for each species (Pérez *et al.*, 2006; Pérez *et al.*, 2008; Table 6.3).

6.2.5. Model initialization and validation

Since all plots used to parameterize the CO_2Fix model were established over former pasture land in which part of the original use is conserved, the average soil organic carbon measured in this part for each species was used to initialize the soil module. Average values of soil organic carbon in the mineral soil up to 30 cm depth for each species studied are shown in Table 6.2. The biomass module was initialized by considering three different site index values for each species (low, medium and high), and stand densities according to the silvicultural objectives (see simulated alternatives, below). This enables construction of a specific yield table for each initial condition.

Validation was carried out for the biomass and the soil module using a network of 120 plots located in afforestations on former agricultural land in northwestern Spain, where carbon density (Mg ha^{-1}) has been evaluated in biomass and soil (Chapter II). Biomass and soil carbon density were estimated by direct measurements in the field, and plotted against values predicted by the CO_2Fix model. Since the model was initialized with the specific site index, initial socking, soil carbon content, and specific climatic data for each one of 120 plots, results show the usefulness of

the model in the region for predicting changes in C accumulation for the tree species studied for afforestation on former pasture land.

Carbon in above and belowground biomass was estimated directly by establishment of circular plots of radius 10 m, in 40 plots of each species considered, in which diameter at breast height and total height were measured. Biomass was calculated from biomass equations at stand level (see point 6.2.2), and values were aggregated at plot level and expressed per hectare. Total carbon in biomass was evaluated from average carbon concentrations observed in each biomass fraction (see point 6.2.2).

Soil carbon density (Mg ha^{-1}) was estimated in mineral soil in each plot, to 30 cm depth and in the soil organic layer. For carbon determination in mineral soil, samples were obtained with a soil corer, on 5 occasions in each plot, considering three layers, 0-5, 5-15 and 15-30 cm, to take into account the logarithmic decrease in carbon concentration with soil depth (Jobbágy & Jackson, 2000). Bulk density samples (Blake & Hartge, 1986) and stoniness were taken into account for soil carbon density estimation with expression [6.8], where *CD* is the carbon density in each layer (Mg ha^{-1}), *CC* is the carbon concentration (as a fraction of unity), *Db* is the bulk density (g cm^{-3}), *T* is the thickness (cm) and *S* is the stoniness (as a fraction of unity).

$$CD = CC \cdot Db \cdot T \cdot (1 - S) \cdot 100 \qquad [6.8]$$

The litter layer was sampled with a 0.3x0.3 m square frame, which was thrown at random on 5 occasions in each plot; all aboveground soil litter within the frame was collected and dried at 40ºC until constant weight. The carbon concentration in the organic layer was used to estimate litter carbon density (Chapter II). Total soil carbon density was estimated as the sum of carbon density in the organic layer and mineral soil to 30 cm depth. Statistical data on biomass, litter and soil carbon densities for each species studied are shown in Table 6.2. To avoid problems related to identifiability of soil compartments in the Yasso model, all were considered jointly for validation and simulation (Palosuo *et al.*, 2006).

Some sensitivity analysis was carried out for the CO_2Fix model (Nabuurs *et al.*, 2008; Palosuo *et al.*, 2008), but there is not much information about the model sensitivity with three crucial parameters in soil C dynamics, i.e. *LT*, *BT* and *RT*. Since an accurate approach based on local empirical models is described here, the model sensitivity for *LT*, *BT* and *RT* was determined. Variations of ±10% and ±20% were simulated relative to the estimation of *LT*, *BT* and *RT* for each species (Table 6.3). This allowed evaluation of the most sensitive parameters relative to the C accumulation in biomass and soil.

6.2.6. Management alternatives simulated

After model parameterization, two alternative silvicultural alternatives were simulated for each species, with the aim of establishing the baseline carbon sequestration for the species in northern Spain when used in afforestation on former pasture land. The alternatives simulated were: A,

aimed at chip-wood production for pulp or board industry, and B, aimed at high quality sawn-wood production. Details about product parameterization for raw material allocation to production lines for all species and alternatives considered are shown in Table 6.4. Site indexes considered for each species were: 17, 21, and 25 m at a reference age of 10 years for *E. globulus* (Fernández López, 1982), 10, 14, and 18 at a reference age of 6 years for *E. nitens* (Pérez-Cruzado, 2009), and 13, 18, and 23 m at a reference age of 20 years for *P. radiata* (Diéguez-Aranda *et al*., 2005).

The chip-wood management alternative (A) for the three species is quite similar in terms of initial stocking and management. This alternative is representative of most *Eucalyptus* plantations in northern Spain, although silviculture for high quality sawn-wood is quite important in *P. radiata* plantations. Initial stocking for alternative A was 1400 stems ha^{-1} for all species, and final cut was simulated at 19, 15 and 12 years for both *E. globulus* and *E. nitens*, and 34, 30 and 28 years for *P. radiata* for each site index respectively. Neither thinning nor pruning was considered for this management alternative.

The sawn wood management alternative (B) starts at the same initial stocking for the tree species considered (1400 stem ha^{-1}), but the management applied was quite different. Since this alternative is not appropriate for poor sites, it was only simulated for the two highest site indexes. Management schemes were similar for both eucalypts, with two thinning at ages 4-3 and 8-6 years (for medium and high site index respectively), with a remaining tree density of 700 and 350 stem ha^{-1} for each intervention. Three prunings were simulated at ages 2-3, 3-4 and 4-5yr (for medium and high site indexes respectively), with pruned heights of 2, 4, and 6 m respectively. Pruning was considered to affect 1000, 800, and 450 stem ha^{-1} in each intervention, and branches and leaves pruned were considered to be incorporated into the soil. These silviculture regimes were based on that proposed by Pérez-Cruzado *et al.* (2011) for *E. nitens*. Clearfelling for alternative B was 20 and 16 yr for the medium and high site index respectively for both *E. globulus* and *E. nitens*.

For *P. radiata*, the sawn-wood management alternative (B) is based on that proposed by Rodríguez *et al.* (2002b). Two thinning were considered at ages 12-10 and 22-20 yr (for medium and high site index respectively), with densities after the operations of 900 and 400 stem ha^{-1} respectively. Two prunings were considered at ages 8-7 and 15-12 yr, with a 1200 stem ha^{-1} pruned until 3 m height in the first intervention, and 500 stem ha^{-1} and affected up to a height of 6m in the second intervention. Clearfelling was set at 35-30 yr for each site index.

Simulations were carried out for the average climate conditions for each species studied (Table 6.1), and initialised with the average soil organic matter content observed in the pasture for each species (Table 6.5). Moreover, two options were simulated for the highest site indexes of each species; harvesting of 50% slash for energy production (considered as default in all simulations), or no slash harvesting. The effect on the reductions in emissions derived from soil and bioenergy were assessed for these two options.

6.3. Results

6.3.1. Biomass compartment validation

The effect of yield tables selected for *P. radiata* stands are shown in Fig. 6.2. It can be seen how both dynamic and static forest-specific models underestimate biomass carbon when high quality afforestation on former agricultural lands is considered. Therefore, a specific model for high quality *P. radiata* plantations established over former agricultural land was fitted. Results of simultaneous fitting provided good results (Table 6.6); all parameters were significant ($p<0.005$) and the adjusted coefficient of determination was similar to those obtained for the models developed for the other two species (Chapter IV). Graphs of observed against predicted biomass C values for *E. globulus* and *E. nitens* plantations are shown in Fig. 6.3. The model prediction works well, because the same plantations were used in developing the models (Chapter IV).

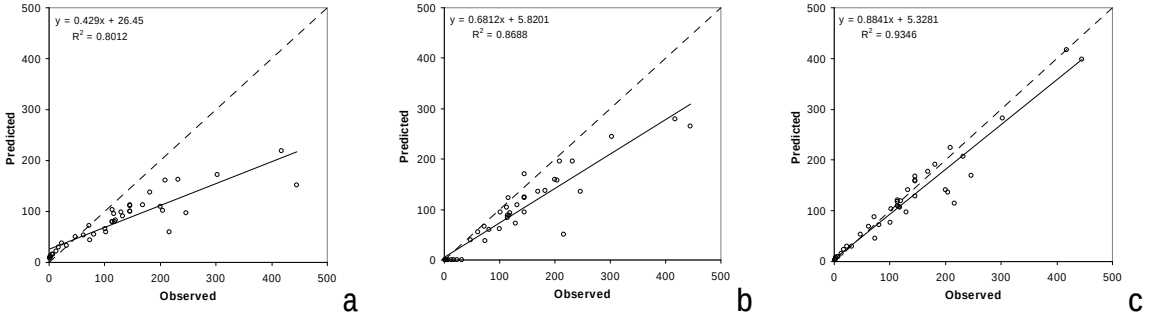

Figure 6.2. Observed against predicted values for aboveground plus belowground biomass carbon in *P. radiata* plots considering: (a) the static growth model developed by Castedo-Dorado *et al*., (2009), (b) the dynamic growth model developed by Castedo-Dorado *et al.* (2007), and (c) a specific model developed for these plots.

Table 6.6. Parameter estimates and model statistics for static yield model for *P. radiata* stands established over former agricultural land.

Parameter	Estimate	*SSE*	*RMSE*	R^2 Adj.
b_0	22.68409	14.9511	3.2833	0.857
b_1	-0.30169	0.1454		
b_2	0.726264	0.0887		
b_3	0.000067	9.37E-6	10.1787	0.999
b_4	2.001942	0.0226		
b_5	0.81586	0.0267		
b_6	0.963125	0.0128		

The results of stand biomass models for biomass allocation factors were also satisfactory. The adjusted determination coefficients were higher than 0.93 for all biomass fractions (Table 6.7), and all parameters were significant ($p<0.05$, Table 6.8). These models were used to determine the biomass fractioning among stem, roots, branches and leaves at each development stage simulated in the yield tables. Data was referred to stem, and set to 1 for implementation in the models.

Table 6.7. Stand biomass equations statistics for determination of allocation parameters.

Fraction	Model	*E. globulus*		*E. nitens*		*P. radiata*	
		RMSE	R^2 Adj.	RMSE	R^2 Adj.	RMSE	R^2 Adj.
W_{tot}	$\sum W_k$	8.5344	0.998	5.7330	0.993	10.6355	0.992
W_s	$b_{k.1} \cdot d_g^{\,b_{k.2}} \cdot H_0^{\,b_{k.3}} \cdot N^{b_{k.4}}$	6.2340	0.998	3.7912	0.992	8.9929	0.996
W_b	$b_{k.5} \cdot d_g^{\,b_{k.6}} \cdot H_0^{\,b_{k.7}} \cdot N^{b_{k.8}}$	0.4201	0.978	1.3534	0.923	1.5227	0.985
W_l	$b_{k.9} \cdot d_g^{\,b_{k.10}} \cdot H_0^{\,b_{k.11}} \cdot N^{b_{k.12}}$	0.0364	0.938	0.1274	0.939	0.0850	0.940
W_r	$b_{k.13} \cdot d_g^{\,b_{k.14}} \cdot H_0^{\,b_{k.15}} \cdot N^{b_{k.16}}$	1.9062	0.958	1.3132	0.956	0.3191	0.960

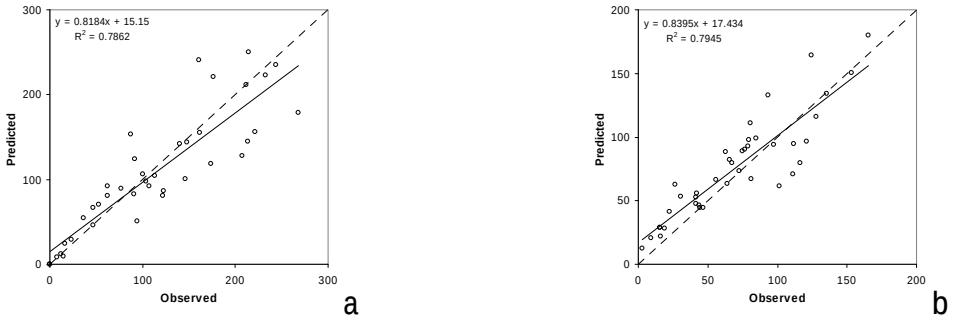

Figure 6.3. Observed against predicted values for aboveground plus belowground biomass carbon in (a) *E. globulus* and (b) *E. nitens* plots, considering the static model (Chapter IV).

Table 6.8. Stand biomass equations parameters for determination of allocation factors.

Parameter	*E. globulus*		*E. nitens*		*P. radiata*	
	Estimate	SSE	Estimate	SSE	Estimate	SSE
$b_{k.1}$	0.00003	5.46E-6	0.000016	1.712E-6	0.000013	1.451E-6
$b_{k.2}$	2.108451	0.0258	2.229016	0.0194	1.876164	0.0299
$b_{k.3}$	0.784985	0.0284	0.76897	0.0166	1.057177	0.0345
$b_{k.4}$	0.9583	0.0175	0.981402	0.0108	1.006	0.0217
$b_{k.5}$	4.363E-6	7.418E-7	0.000013	2.484E-6	0.000023	7.514E-6
$b_{k.6}$	2.48314	0.0245	2.342609	0.0378	2.117443	0.0416
$b_{k.7}$	0.097557	0.0264	0.250399	0.0320	0.099399	0.0352
$b_{k.8}$	1.039028	0.0160	0.979774	0.0205	1.009958	0.0334
$b_{k.9}$	0.000031	2.717E-6	4.739E-6	3.949E-7	0.000043	6.508E-6
$b_{k.10}$	1.921303	0.0130	2.302002	0.0158	1.73639	0.0200
$b_{k.11}$	-0.01404	0.0139	0.341281	0.0134	-0.0259	0.0169
$b_{k.12}$	0.995307	0.00894	0.989221	0.00855	0.994896	0.0155
$b_{k.13}$	0.000013	1.864E-6	3.98E-6	4.246E-7	0.000062	2.012E-6
$b_{k.14}$	2.126404	0.0209	2.24939	0.0196	2.129126	0.00410
$b_{k.15}$	0.700717	0.0230	0.660225	0.0167	0.014479	0.00344
$b_{k.16}$	0.965065	0.0142	0.980309	0.0108	1.001264	0.00332

6.3.2. Soil compartment validation

Soil C densities estimations showed a strong bias towards underestimation, results of observed-predicted values for the validation plots are shown in Fig. 6.4. Estimation for all species studied followed the same pattern, with estimated values similar to observed values for the lower initial carbon contents and much lower than the observed values for the highest carbon contents. The variability in *P. radiata* was lower than for both *Eucalyptus* spp., although the same trends were observed for all species in the graphs of observed-predicted values. For punctual estimation of C accumulation in soil, the observed-predicted correction models shown in Fig. 6.4 can be used.

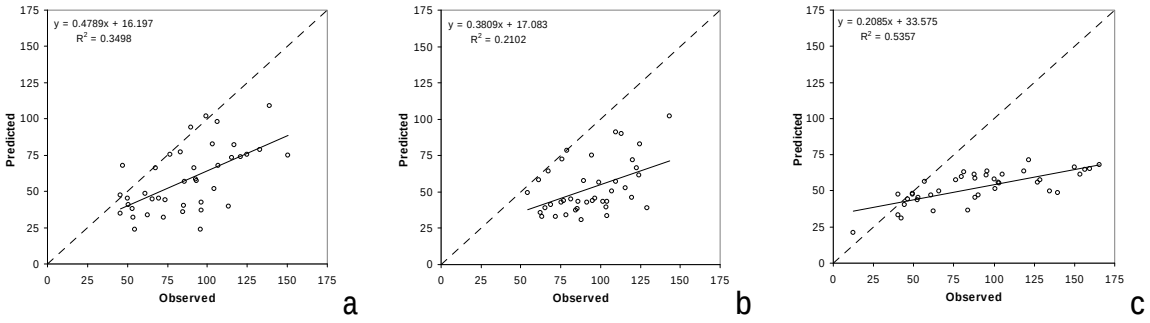

Figure 6.4. Observed against predicted for soil carbon (all compartments) in (a) *E. globulus*, (b) *E. nitens*, and (c) *P. radiata* plots.

6.3.3. Sensitivity analysis

The sensitivity analysis for the leaves, branches and roots turnover is shown in Fig. 6.5. The Yasso model displayed low sensitivity as regards these parameters, as increments of ±20% in each parameter supposes differences in soil carbon accumulation <0.5% for the 120 plots used for model validation. The model displayed higher sensitivity to root turnover than to branch or leaf turnover.

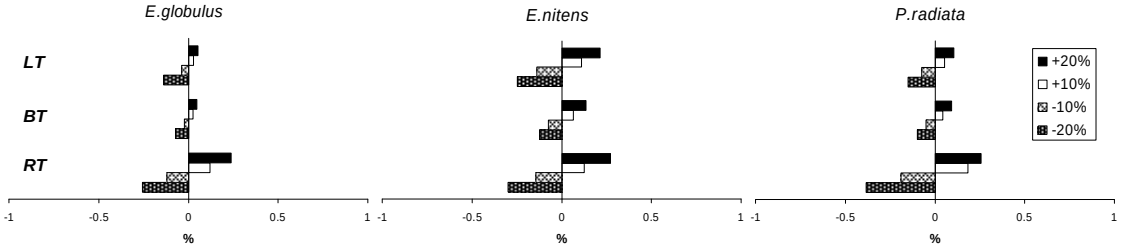

Figure 6.5. Sensitivity analysis for leaf (*LT*) branch (*BT*) and root (*RT*) turnover for the three species studied.

6.3.4. Model simulation

Outputs of the parameterized model are shown in Figs. 6.6-6.8. These figures show the changes in C stocks over several rotations in the same stand. Assumptions for these outputs imply that yield tables are suitable for all rotations considered, which would be reasonable if no coppice rotations were considered. Another important assumption is that climate will remain unchanged in the study period.

Although there were large oscillations among alternatives, a new equilibrium was reached after afforestation of former agricultural land with the tree species studied for some compartments, namely biomass, products and soil (Figs. 6.6-6.8). This implies average C stocks in the stand biomass after land use change (*BS*) of 41.1, 52.9 and 66.0 Mg C ha^{-1} for all alternatives simulated for *E. globulus*, *E. nitens* and *P. radiata* respectively.

Equilibrium C accumulation in products (*PS*) became an important carbon sink after land use change. It followed a similar shape as observed for *BS*, and tended to stabilize as time since afforestation increased. The average *PS* values after land use change were 91.9, 156.0 and 95.0 Mg C ha^{-1} for all alternatives simulated for *E. globulus*, *E. nitens* and *P. radiata* respectively (Fig. 6.7).

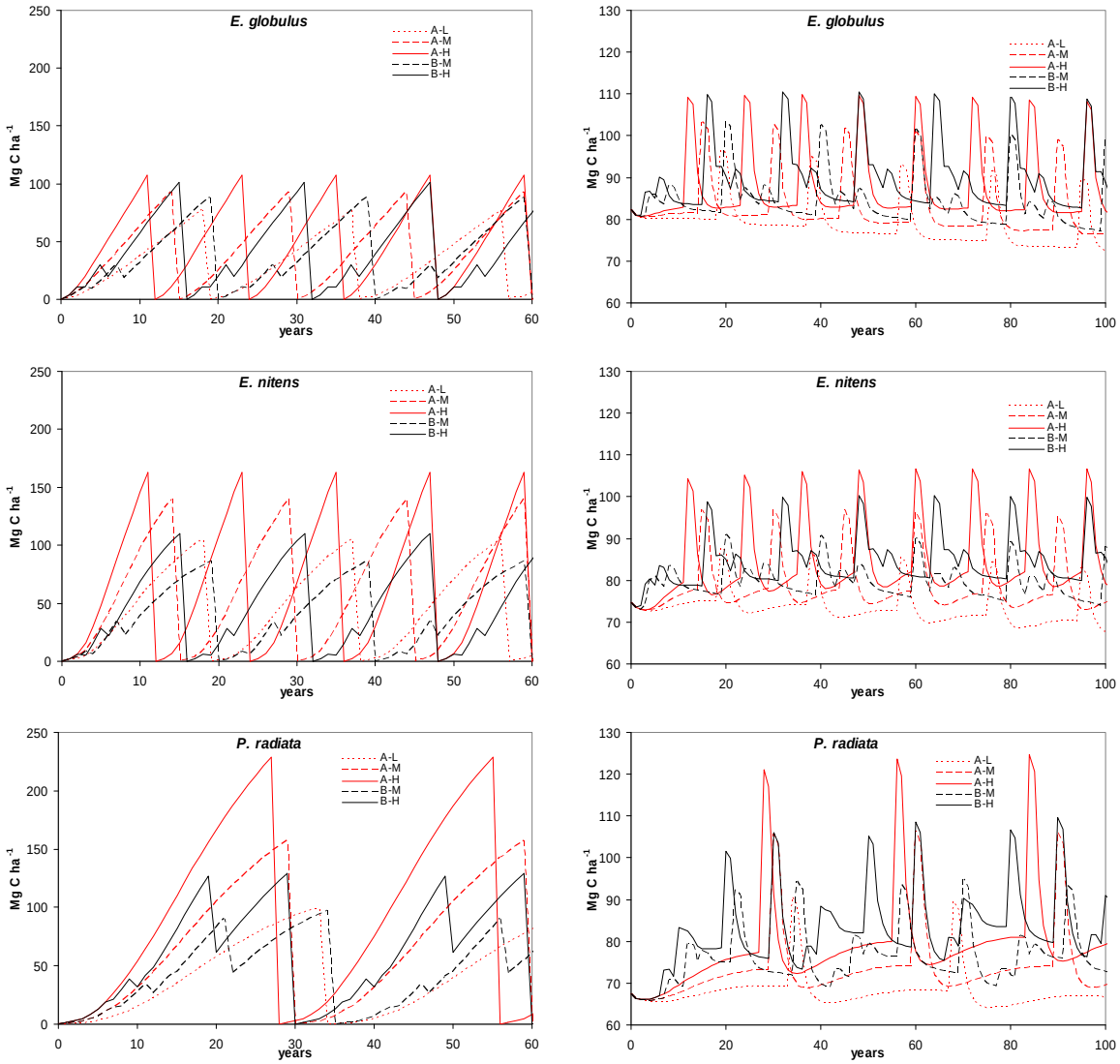

Figure 6.6. Model simulations for the stand biomass (left) and soil (right) for the two silvicultural alternatives and site indexes considered for each species.

As regards soil, simulations of the alternatives considered are shown in Figs. 6.6 and 6.8. The overall change after 100 years for all alternatives simulated was a slight increase in C density in *P. radiata* stands, whereas in both species of *Eucalyptus* the values remained more constant or even decreased for the lowest site index. The C stock equilibrium values in the soil after land use change (*SS*) were 80.6, 77.8 and 76.9 Mg C ha^{-1} for all alternatives simulated for *E. globulus*, *E. nitens* and *P. radiata* respectively, considering harvesting of 50% slash for bioenergy. The average value for each compartment considered (*BS*, *PS* and *SS*) varied depending on the age at which the steady state was considered for each compartment. Thus, the 0-200 yr period was considered for *BS*, 125-200 yr for *PS* and 50-200 yr for *SS*. The C stock values for each species and management alternatives are shown in Table 6.9.

For all alternatives simulated, the average *BS* values decreased in the order *P. radiata* > *E. nitens* > *E. globulus*, the average *PS* values decreased in the order *E. nitens* > *P. radiata* > *E.*

globulus, and the *SS* values decrease in the order *E. globulus* > *E. nitens* > *P. radiata*. Although there were large differences in productivity among the species studied, the simulation results did not differ as regards the order of the three species studied for the initial SOC content in the pasture (Table 6.2) relative to the *SS* previously reported.

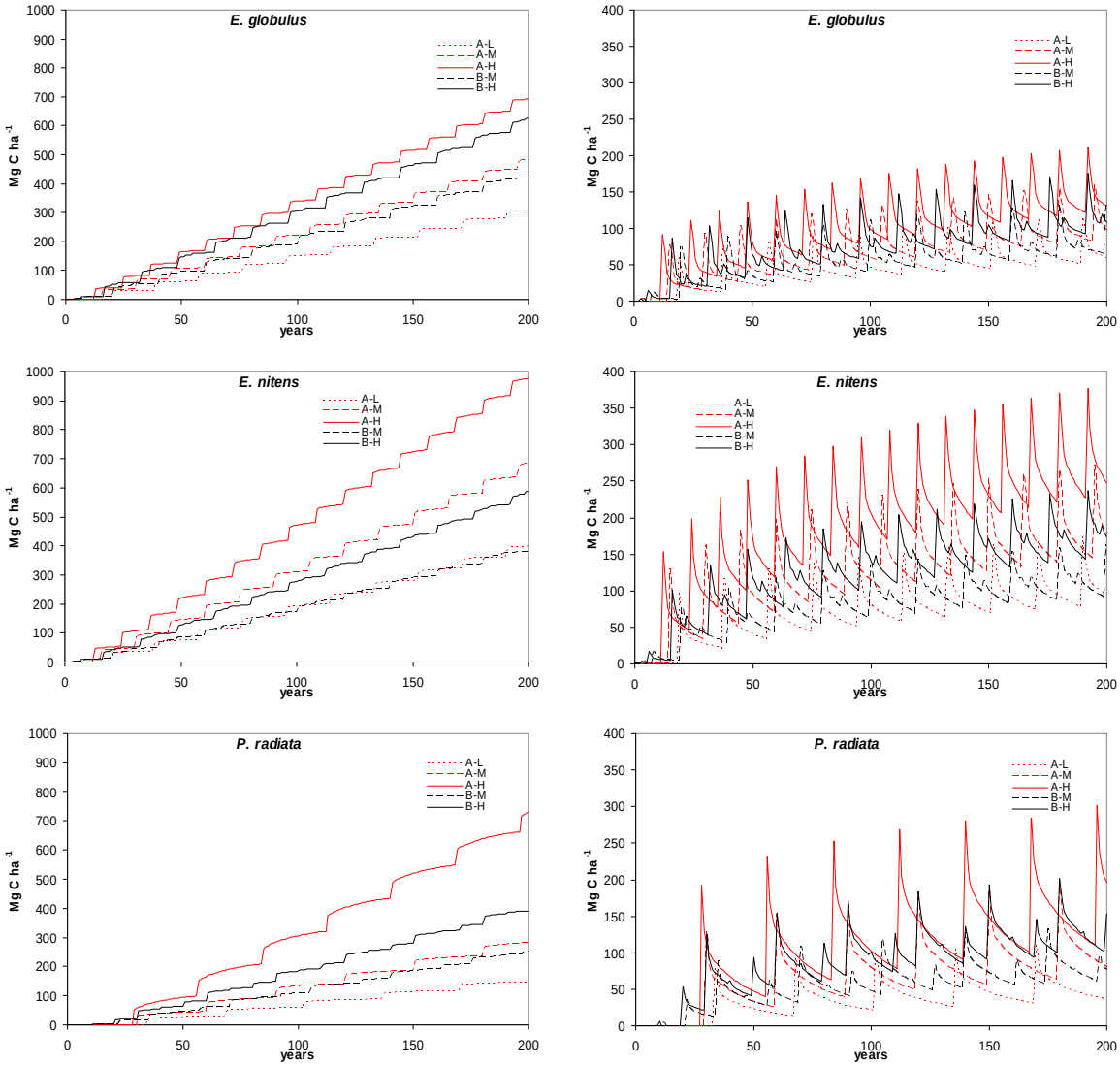

Figure 6.7. Model simulations for products (right) and bioenergy (left), for the two silvicultural alternatives and site indexes considered for each species.

Because the mitigation effect occurs immediately after bioenergy use, representations in the CO_2Fix model are accumulated avoided emissions (Figs. 6.7 and 6.8). This makes it difficult to make comparisons with stock changes that occur in other compartments, as previously reported. The avoided C emissions resulting from bioenergy use (*BM*, Mg C ha^{-1} yr^{-1}) were therefore estimated for each alternative and site quality considering a period of 200 years after land use change (Table 6.6). The most effective species for *BM* when harvesting of 50% slash residues is considered is *E. nitens*, followed by *E. globulus* and *P. radiata*. When harvesting of residues is not considered, both *Eucalyptus* showed similar value of *BM*, double than observed for *P. radiata*.

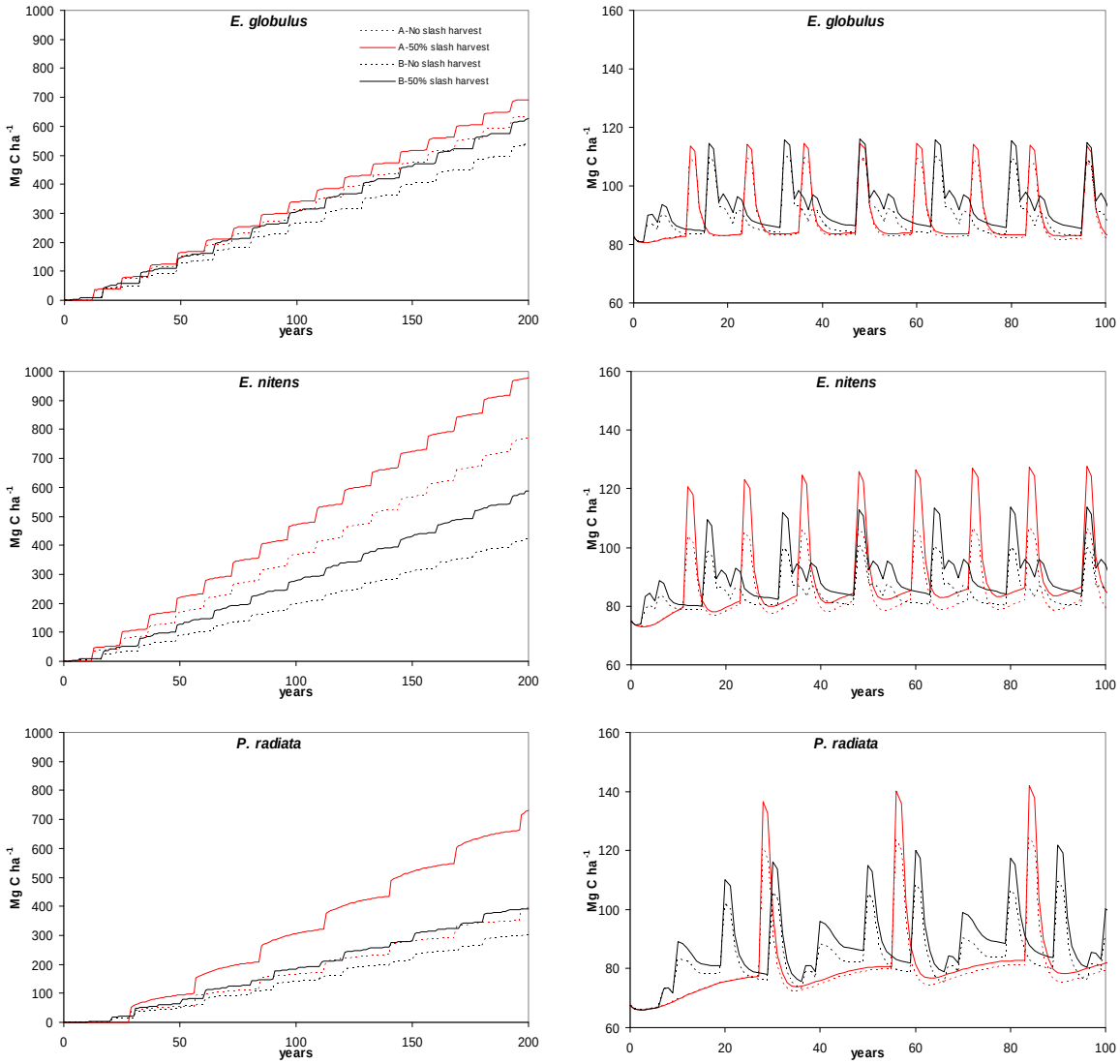

Figure 6.8. Model simulation alternatives A-H (red) and B-H (black), considering 50% slash removal for bioenergy (continuous line) or no slash removal (dotted lines). Left: bioenergy substitution; right: soil.

6.3.5. Effect of simulated management alternatives

Management alternatives are compared in Figs. 6.6-6.7 and two biomass extraction methods for bioenergy use are compared in Fig. 6.8. Results of average C stock in the steady state after land use change (*BS*, *PS* and *SS*) and average avoided emissions by energetic use (*BM*) are summarized for each alternative and species in Table 6.9.

Management alternative A (chip-wood) is more effective, for *BS*, *PS* and *BM*, relative to alternative B (sawn-wood, Table 6.9). Site quality played an important role in this aspect, with differences among the same alternative for the C stock in *BS* as high as ±19.8%, ±18.8% and ±48.6% for alternative A, and ±8.2%, ±11.0% and ±27.6% for alternative B, for *E. globulus*, *E. nitens* and *P. radiata* respectively (Table 6.9), which shows that the equilibrium C stock in

biomass is more sensitive to site index for management alternative A than for alternative B. Management alternatives A and B were therefore compared by considering only the two highest site indexes. On the other hand, alternative A was less effective than alternative B as regards *SS* for all species considered.

Table 6.9. Simulation outputs for each management alternative.

	A-L	A-M	A-H	B-M	B-H	TOTAL
BIOMASS (Mg C ha^{-1})						
E. globulus	34.2 (25.2)	41.5 (30.4)	49.1 (34.8)	38.8 (27.4)	42.0 (32.7)	41.1 (30.5)
E. nitens	48.1 (36.7)	60.3 (48.6)	67.7 (55.4)	41.8 (28.7)	46.4 (38.4)	52.9 (43.4)
P. radiata	48.4 (35.1)	64.6 (50.4)	106.0 (78.9)	48.9 (31.0)	62.4 (41.2)	66.0 (54.3)
SOIL: 50% SLASH HARVEST (Mg C ha^{-1})						
E. globulus	72.5 (6.3)	78.1 (7.7)	85.2 (9.5)	79.8 (6.7)	87.4 (8.0)	80.6 (9.3)
E. nitens	68.8 (5.1)	76.2 (7.0)	83.7 (9.3)	76.4 (4.5)	84.0 (5.9)	77.8 (8.6)
P. radiata	65.5 (5.7)	73.6 (8.7)	83.0 (12.3)	76.1 (6.8)	86.6 (9.0)	76.9 (11.5)
SOIL: NO SLASH HARVEST (Mg C ha^{-1})						
E. globulus	73.4 (7.0)	79.6 (8.7)	87.4 (10.9)	82.9 (7.4)	92.0 (8.8)	83.1 (10.7)
E. nitens	72.1 (7.6)	81.9 (11.2)	91.5 (15.0)	82.1 (6.4)	92.5 (8.6)	84.0 (12.6)
P. radiata	67.2 (7.2)	76.5 (11.4)	87.5 (16.4)	80.8 (8.6)	93.5 (11.5)	81.1 (14.6)
PRODUCTS (Mg C ha^{-1})						
E. globulus	59.7 (16.7)	91.6 (22.2)	132.6 (27.4)	71.9 (18.0)	103.9 (22.9)	91.9 (33.5)
E. nitens	93.9 (23.7)	164.7 (34.5)	251.8 (43.0)	104.6 (18.7)	165.1 (26.5)	156.0 (64.0)
P. radiata	47.0 (16.4)	90.5 (25.9)	146.7 (46.0)	72.9 (16.7)	117.8 (23.0)	95.0 (44.4)
BIOENERGY: 50% SLASH HARVEST (Mg C ha^{-1} yr^{-1})						
E. globulus	1.4 (0.2)	2.2 (0.3)	3.2 (0.4)	1.9 (0.4)	2.8 (0.6)	2.3 (0.7)
E. nitens	1.8 (0.2)	3.0 (0.4)	4.4 (0.6)	1.7 (0.3)	2.5 (0.5)	2.7 (1.1)
P. radiata	0.7 (0.1)	1.2 (0.2)	3.0 (0.5)	1.0 (0.3)	1.7 (0.4)	1.5 (0.9)
BIOENERGY: NO SLASH HARVEST (Mg C ha^{-1} yr^{-1})						
E. globulus	1.3 (0.2)	2.0 (0.2)	2.9 (0.3)	1.6 (0.4)	2.4 (0.5)	2.0 (0.6)
E. nitens	1.4 (0.2)	2.4 (0.3)	3.4 (0.5)	1.2 (0.3)	1.8 (0.4)	2.0 (0.9)
P. radiata	0.5 (0.1)	1.0 (0.2)	1.6 (0.3)	0.8 (0.2)	1.3 (0.3)	1.0 (0.4)

The proportion of harvested slash affects *SS*, but also *BM*. The *BM* increased and *SS* decreased with increasing proportion of slash harvesting (Figs. 6.6-6.8, Table 6.9). Thus, harvesting 50% of slash residues resulted in increases in *BM* of +9.3%, +27.7% and +49.2% for alternative A, and of +17.8%, +40.3% and +27.9% for alternative B, for *E. globulus*, *E. nitens* and *P. radiata* respectively, whereas the predicted decreases in *SS* were -1.9%, -6.7% and -3.8% for alternative A, and -4.4%, -8.0% and -6.6% for alternative B, for *E. globulus*, *E. nitens* and *P. radiata* respectively (Table 6.9).

6.4. Discussion

6.4.1. Dependence on base-models and turnover assumptions

The intrinsic stochasticity of yield model parameters implies high variability between forests, and even within a clearly defined forest type because of annual variations in weather conditions, genetic differences and variations in site quality (Nabuurs *et al.*, 2008). Differences in observed-predicted biomass carbon (Figs. 6.2-6.3) are therefore not due to the CO_2Fix model, but to the representativeness of the chosen models. Although the precision of selected forest-specific models for *P. radiata* have been found to be good enough for forest land (Castedo-Dorado *et al.*, 2007; Castedo-Dorado *et al.*, 2009), the results obtained here demonstrated a slight bias when these models were applied to forest plantations established on former agricultural land. This may be because of the higher average site quality observed in the plots relative to those used to fit the models. The dynamic model (Castedo-Dorado *et al.*, 2007) worked better than the static model (Castedo-Dorado *et al.*, 2009), but the best estimates were obtained with the afforestation specific static models (Figs. 6.2-6.3).

CO_2Fix biomass module estimations have also been validated for biomass compartment by other authors (Masera *et al.*, 2003), who reported underestimation of biomass, which they attributed to the fact that the model input data represented managed forest, whereas the published data represented undisturbed forests. Since CO_2Fix relies heavily on net annual increment from yield tables (Nabuurs *et al.*, 2008), the user should chose adequate yield tables. This is an important point to be considered when CO_2Fix is used for scaling up from stand level to landscape or higher level, where selected sites should represent the average growth conditions of tree species considered and the selected climate zone (Karjalainen *et al.*, 2002). In such cases, this approach may guarantee the representativeness of the yield tables for the scale considered. An average site index weighted by the surface may be appropriate for obtaining the representative yield tables for a given region.

On the other hand, simulations carried out on this work considered invariable climatic conditions in the study period for both validation and evaluation alternatives. The main constraint of all empirically based models is that their applicability is restricted to the scope of the original data (Korzukhin *et al.*, 1996), including the climatic conditions.

Results for soil module validation showed that the estimated values were close to observed values for the lowest carbon densities, whereas the highest carbon densities were greatly underestimated (40-52%, Fig. 6.4). A trend in these same plots with age was observed in Chapter II, where the youngest plots had the lowest carbon densities and the oldest the highest carbon densities. Bias in model outputs were therefore due to overestimation of decomposition rates for the model, which led to greater differences as time since land use change increased. This effect has also been noted by other authors who parameterized the Yasso model for *Eucalyptus* spp.

(Lemma *et al.*, 2007) and for other species (de Wit *et al.*, 2006; Palosuo *et al.*, 2008). This is attributed to overestimation of decomposition of soil compartments, but also because the model does not consider litter input sources other than trees.

In the CO_2Fix model, the effects of climate on decomposition are related to the macroclimate, and some effects of microclimatic conditions on SOC decomposition may not have been taken into account. Lemma *et al.* (2007) considered that the contribution that different species make to microclimate is small relative to litter input and litter quality, although in Chapter II it is suggested that these differences may be greater. Large differences among species in soil organic carbon evolution after land use change were also observed in the same plots as those used here for validation, which only can be attributed to species induced differences in microclimatic conditions (Chapter II).

Nabuurs *et al.* (2008) carried out a sensitivity analysis for the CO_2Fix model for temperate and tropical forests and concluded that stem parameters (CAI, density and carbon content) and initial carbon stock on humus type 2, largely determine the outcome of the model. These authors explained this as being because larger amounts of C are stored in stems and humus type 2 than in other fractions (roots, foliage, branches and other soil compartments), and therefore recommend paying more attention to stem parameter model parameterization. However, the model sensitivity relative to other parameters like *LT*, *BT*, and *RT* is still not clear (de Wit *et al.*, 2006). Moreover, these parameters are not always available, which may lead to too many assumptions and simplifications. A detailed approach is described for *BT* and *RT* determination from species-specific allometric relations, whereas *LT* is estimated from reported values of 3-PG parameters for the species studied.

Turnover parameter estimations (*LT*, *BT* and *RT*) for the studied species are consistent with those observed for other authors for several species (Schelhaas *et al.*, 2004). With regard to *LT*, parameter values imply a mean leaf lifespan of 3.1, 3.9 and 2.8 yr for *E. globulus*, *E. nitens* and *P. radiata* respectively, which are considered reasonable for adult leaves of both *Eucalyptus* and also for *Pinus*. The *BT* values are also in the order of reported by Schelhaas *et al.* (2004) for several species worldwide. Root turnover is difficult to determine because not many studies have been carried out, and the values vary substantially depending on the methodology used. Jourdan *et al.* (2008) observed differences as great as 0.7-1.8 $year^{-1}$ depending on methodology used for *Eucalyptus* plantations in Brazil. The root turnover rate is generally greater than that of leaf turnover (Swift *et al.*, 1979). This parameter takes the same value (0.18 yr^{-1}) for the tree species in reported 3-PG parametrizations (Rodríguez *et al.*, 2002a; Sands & Landsberg, 2002; Pérez-Cruzado *et al.*, 2011), but different values are obtained for each species considering the specific sizes distribution and turnover rates in the fine and coarse fraction (Table 6.3).

Sensitivity analysis for the effects of *LT*, *BT* and *RT* on soil C estimation showed that the Yasso model is strongly robust with regard to these parameters. All showed deviations <±0.5% with variations of ±20% in the turnover parameter. The robustness of the Yasso model in comparison with alternative soil C models has been studied for other parameters by (Palosuo *et*

al., 2008), who concluded that Yasso model is more conservative in the estimations of changes in SOC after input of fresh organic matter, because of higher decomposition estimation. Moreover, *RT* was found to be the most sensitive parameter, probably because root turnover is the most important source of organic matter to mineral soil in temperate forests (Rasse *et al.*, 2001).

Uncertainty in estimates obtained by the CO_2Fix model has been found to depend on data availability (Nabuurs *et al.*, 2008). The uncertainty remains very high, even when there is good access to the data, and much higher than can reasonably be achieved in carbon sequestration through changes in forest management. The main sources of uncertainty are that: (i) stem parameters largely determine model outputs, (ii) depending on the initial state of the model, perturbation can lead to multiple equilibria, and (iii) the standard deviation of total carbon stock is twice as high in tropical secondary forest than in temperate forest because of wood density and current annual increment (Nabuurs *et al.*, 2008). Another strong source of uncertainty is the mortality rate in tropical forests, which can be 10 times greater than in temperate managed forests (Nabuurs *et al.*, 2008).

Thus, for the 95% confidence interval the average values ranged ±24% when data availability was high, and ±86% when data availability was low for the biomass and soil compartments (Nabuurs *et al.,* 2008). A similar degree of uncertainty was observed in CO_2Fix (95% confidence levels after 90 years amounted to ±20%) and was considered quite large (Groen *et al.,* 2006). Another national study, in Finland, reported lower levels of uncertainty for the forest and soil (Monni *et al.*, 2007). For direct measurement of the effect for chronosequences and pairwise plots, in the same validation plots in the present study, 95% confidence intervals ranged from ±16.6% and ±18.0% only for the soil compartment (Chapter II). The bias in the soil estimation is therefore considered a more serious risk than the uncertainty when applying the CO_2Fix model.

Nabuurs *et al.* (2008) observed that the soil made the smallest contribution to the overall uncertainty. They attributed this to the low number of parameters and the hidden temperature sensitivity functions in the model, which makes it more robust (Nabuurs *et al.*, 2008). The key uncertainty in the soil sink with the Yasso model is the initial value of soil C stock (de Wit *et al.*, 2006), because if soil C stock is not in the steady state relative to decomposition for the climatic conditions simulated, the model may report erroneous trends. One way of correcting this is to run the model until it reaches the steady state. However, this source of uncertainty is reduced in importance when approaching the end of the data series (de Wit *et al.*, 2006).

This was corrected in the present study by considering the average carbon content during the previous land use (pasture) as the average value for the 40 pairwise plots measured for each species considered. Since changes in C in the soil compartment after simulations did not show strong oscillations in the medium term, this approach was considered adequate. As previously reported, other significant sources of uncertainty are the rates of turnover of roots and foliage (de Wit *et al.*, 2006), although in the present study high data availability enabled accurate estimation.

6.4.2. Mitigation effect of several management alternatives

When evaluating the whole forest system, there are great uncertainties as regards the persistence of the sink and how these temporary sinks should be considered. One attempt to do this is by the advancing mean method described by Nabuurs & Schelhaas (2002). On this paper, the average C stock in the steady stage (*BS*) was evaluated as the average in the period 0-200 yr for the biomass.

Evaluation of the effect of afforestation of former pasture land on C accumulation was applied in the present study considering the C stock on biomass on pasture lands to be 0. The highest *BS* was reached in *P. radiata* stands because of the longer rotation age, which enabled an average C stock of 66.0 Mg C ha^{-1} to be reached. Management alternative A produced larger C stocks for all species considered than alternative B, because of biomass reduction by thinning (Fig. 6.6; Table 6.9). These results are consistent with those of Mund & Schulze (2006), who reported that the total amount of carbon in the system decreased as management intensity increased in *Fagus sylvatica* stands in Germany. The same effect was reported by Matala *et al.* (2009), who observed that future evolution of C in the growing stock depends on forest management, as well as on climate change scenarios. Moreover, *BS* increased with site quality for all species studied.

Changes in soil C stock were found to depend on the management alternative and site index considered (Figs. 6.4, 6.8; Table 6.9). Initial C losses were observed in all simulations in the first 8 years. This effect was reported by other authors using the same methodology (Nabuurs & Schelhaas, 2002) or using empirical data from the same plots (Chapter II), and is considered to be a result of ignoring the herbaceous root organic matter input to the soil.

The present results showed that changes in land use have a greater impact on biomass carbon stock than on soil stock, as also reported by other authors (de Wit *et al.*, 2006), although soil C is more stable than biomass C. There were slight differences in *SS* in the simulated period (50-200 yr) for the different site indexes and management alternatives considered. The *SS* increased with site index for all species. Alternative B produced highest C stock in the steady state, because more organic matter was incorporated to the soil in pruning and thinning operations (completely or 50% of residues were left in place). The Yasso model also shows low sensitivity to changes in OM input to the soil than other models (Palosuo *et al.*, 2008), which make the predictions more conservative. It is therefore possible that real differences among alternatives would be greater than predicted here.

The most important factors involving SOC accumulation in afforestation over former agricultural land are changes in: (i) litter production, (ii) litter quality, (iii) the depth to which organic matter is incorporated, (iv) the microclimatic conditions, and (v) the physical and chemical stability of SOM (Lugo & Brown, 1993; Post & Kwon, 2000; Paul *et al.*, 2002; Lal, 2005). Some process-based models are sensitive to these parameters, but the Yasso model is only sensitive to climatic conditions, organic matter quality, organic matter input and initial conditions (Liski *et al.*, 2005) and therefore, only conclusions derived from these effects can be reached. Moreover, microclimatic

conditions have been found to affect the changes in SOC in relation to this land use change (Chapter II), although this was not considered in the Yasso model.

Although inclusion of ground vegetation in this type of analysis is not usual, its inclusion may provide more realistic estimation of SOC after land use change. Because ground vegetation recovers rapidly after thinning and clearcuting, its effect on SOC may result in smoothing of the simulated changes in the soil compartment (Palosuo *et al.*, 2008). Differences among tree species in *SS* were also observed by using the CO_2Fix model to describe the same land use change as studied here (Lemma *et al.*, 2007).

As it is not yet clear how carbon in wood products will be credited, it is important to compare the total outcome of the scenarios with and without products (de Jong *et al.*, 2007). If products are not considered, all C in products is taken into account as net emission at harvest. If products are considered, the balance between new products being produced and old products being decomposed, may also provide a net sink in products-in-use (Nabuurs & Schelhaas, 2002). Thus, a new pool appears with forest product use, with a double mitigating effect: by carbon sequestering in its own matter, and by substituting alternative products for which fossil C releases are needed. For this hypothesis to hold true, it must be proved that the C balance in wood product use is more favourable than natural mortality processes. Profft *et al.* (2009). reported that the mean residence time is higher in wood products than in dead wood for the conditions in central Germany, which implies that use of wood products results in a carbon sink that otherwise would not exist

Carbon in forest products was considered as an additional pool in the present study, and average C stock in the steady state (*PS*) was calculated for the period 125-200 yr after land use change. Under the assumptions of product lifespan considered (Table 6.5), the total C stock in forest products is greater than those in biomass (Table 6.9). Site index had an important effect on product stock, with *PS* increasing with site index. In comparison with other studies, De Jong *et al.* (2007) observed similar C stocks in the product pool (40-65 Mg C ha^{-1}) in the oak forest of Central Highlands of Michoacán (Mexico).

Although some authors have observed that low forest utilization yielded more forest products than the high utilization system (Harmon & Marks, 2002), the results of the present study demonstrate that alternative A generates more C stock in the products than alternative B. This may be due to the difference in the assumptions regarding lifespan, since a larger proportion of long-term products are generated in management alternative B than in alternative A. Other authors considered lifespan periods for the different product groups of 50, 16, 4 and 1 yr for long, medium-long, medium-short and short life span products respectively (Row & Phelps, 1990; Karjalainen *et al.*, 1994; Karjalainen, 1996; Nabuurs & Sikkema, 2001; Karjalainen *et al.*, 2002). The assumptions used here as regards the product lifespan can be considered as conservative, and *PS* may actually be larger. Silviculture also affects the lifespan of harvested products, for instance, thinning from above leads to a mean residence time of 23 yr, and thinning from below to a residence time of 18 yr (Profft *et al.*, 2009). The latter author reported an average residence time in wood products of 20 yr, which was the longest life-span considered in the study.

On the other hand, not all this *PS* can be considered directly as avoided emissions, since there are additional releases from fossil fuel sources in the delivery chain and industrial processes. In a recent study on fuel consumption in road transport of round wood in Austria (Holzleitner *et al.*, 2010), the emission per cubic meter of roundwood due exclusively to transport was 2.23 kg C m^{-3}, considering an average lorry load of 25 m^3 and an average distance (forest-industry) of 51 km. This emission must be discounted from the C gain derived from use of wood products.

Other forest products must be considered as flux rather than stock, i.e. bioenergy production. The mitigating effect of bioenergy production (*BM*) is the only strictly long term mitigating potential of forest systems (Lindner & Karjalainen, 2007), as the other pools considered reach equilibrium after land use change, and the accumulation effect becomes limited. *BM* was higher in alternative A than in alternative B. This follows the same trend as *PS*, because some of the energy use occurs with the log-wood and pulp-wood waste and recycling processes, and the other part occurs with the slash. Values for *BM* obtained in this study are higher than those reported by de Jong *et al.* (2007) for the bioenergy scenario, which resulted in continuous stream of about 1.36 Mg C ha^{-1} yr^{-1}, even in the case of no slash harvesting. The effect for site index was the same as observed for the other compartments, i.e. the higher the site index the greater mitigation rate by bioenergetic use.

The effects of the intensity of slash harvesting on *BM* are shown in Table 6.6, with increments of between 17.8% and 40.3% occur by harvesting 50% of slash relative to no slash harvesting. On the other hand, harvesting 50% of slash results in decreases of between 1.9% and 8.0% of soil C equilibrium stock. This effect has also been observed by other authors (Johnson & Curtis, 2001). Some studies have shown that the gain in SOC obtained by leaving harvesting residues in the field is stronger in coniferous than in broadleaved species (Hendrickson *et al.*, 1989; Mattson & Swank, 1989; Knoepp & Swank, 1997; Johnson & Todd, 1998), although some studies on coniferous forests have also shown little or no effect of residues on soil C or N (Olsson *et al.*, 1996). The greatest effects on both *SS* decreases and in *BM* increases were in *E. nitens*, probably because of its high yield and crown biomass (Chapter IV).

Studies of the global carbon cycle in relation to bionenergy systems conclude that although there may be some decrease in soil carbon associated with biomass production, this is negligible in comparison with the contribution of bioenergy systems towards greenhouse mitigation by avoiding fossil fuel emissions (Cowie *et al.*, 2006). Moreover, the effect of harvesting residues on SOC is time limited, because C and N increase temporally because of residues becoming incorporated into the soil (Smethurst & Nambiar, 1990; Black & Harden, 1995; Knoepp & Swank, 1997), and the increase in soil C and N is short-lived (less than 4 years, according Smethurst and Nambiar, (1990)). Nevertheless, some authors have reported that intensified biomass extraction slightly decreased forest growth and thus the biomass carbon stock and litter input to the soil (Palosuo *et al.*, 2008; Helmisaari *et al.*, 2011).

Logging residues left in place after first thinning have been found to be greatly influenced by the top diameter threshold considered (Räisänen & Nurmi, in press), and slash production was

almost doubled, with increments of 2 cm in the top diameter threshold. With the slash harvesting machinery currently used, it seems unlikely that more than 50% of harvest residues will be collected (Tolosana, per. com.). According to the results obtained in the present study, a combination of bioenergy and carbon sequestration will be the best option for mitigating CO_2 emissions in the long term, as already reported for plantation management in other countries (de Jong *et al.*, 2007; Kaul *et al.*, 2010).

6.5. Conclusions

Yield tables have a major impact on CO_2Fix output estimations, even when local growth models are available. When afforestation on former agricultural land is simulated, specific models for these conditions are required.

The Yasso model underestimates the C content under the conditions described here. This is due to overestimations in the decomposition rates of the soil compartments.

Site index is an important factor in changes in system carbon, with variations between ±12% to ±64% for biomass, ±9% to ±13% for soil, ±44% to ±62% for products, and ±45% to ±150% for bioenergy substitution.

A 50% reduction in thinning and clearfelling slash harvesting results in a 17.6%-40.3% increase in the bioenergy mitigating effect, whereas soil equilibrium C stock decreased by 4.4-8.0% relative to no slash harvesting.

In the conditions described here, the silvicultural alternative aimed at chip-wood production was the most effective in storing carbon in biomass, products and bioenergy, whereas the alternative aimed at sawn-wood production was more effective for storing carbon in the soil. The latter effect was due to the more frequent organic matter input to the soil via pruning and thinning waste.

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Chapter VII

General discussion and conclusions

7. General discussion and conclusions

The main results presented in Chapters II-VI are discussed jointly here. To avoid repetition of contents, three main topics are discussed as follows: (i) the effect of the afforestation of former pasture land on carbon accumulation in several forest compartments, (ii) the effect of different estimation approaches on the accuracy of carbon estimation in forest stands, and (iii) the effect of different management practices on carbon accumulation in forests.

7.1. Effect of afforestation of former pasture land on carbon accumulation

7.1.1. Tree biomass

Changes in biomass and carbon accumulation in biomass were evaluated from the average changes in the measured plots (Chapter II), and models were developed for biomass accumulation at tree level for *E. nitens* (Chapter III), and at stand level for *E. globulus* and *E. nitens* (Chapter IV) and *P. radiata* (Chapter VI).

The average changes in biomass C for the chronosequences in plantations established on former pasture land are shown in Figs. 2.1 and 2.6. Of the species considered, *E. nitens* was the most effective for sequestering C in biomass, followed by *E. globulus* and *P. radiata*. The mean annual increments were higher than the productivity recorded for the plantations established on former forest soils in the region (Balboa-Murias *et al.*, 2006). The enhanced growth is attributed to the greater soil depth and availability of nutrients derived from previous fertilizations in the pasture soils.

Nevertheless, the highest biomass and carbon densities were reached in the *P. radiata* plots, at the end of the rotation. Moreover, simulation studies (Chapter VI) demonstrated that higher average carbon density in biomass up to 200 years was reached in *P. radiata* stands than in *Eucalyptus* stands. This is because of the longer rotation length of *P. radiata* stands than of *Eucalyptus* stands. The proportion of biomass in each biomass fraction at the end of the rotation was 4% for leaves, 8, 23 and 15% for branches, 77, 64 and 68% for wood and 11, 9 and 13% for bark, for *E. globulus*, *E. nitens* and *P. radiata* respectively.

Biomass yields for slightly higher densities than usual in forestry were evaluated (Chapter IV) for *E. globulus* and *E. nitens*, although the initial stocking densities were much lower than usually reported for short rotation woody crops. Yields of *E. globulus* were similar to those reported by

several authors for much higher densities (Cromer *et al.*, 1975; Pereira *et al.*, 1994; Guo *et al.*, 2002), but the predicted *E. nitens* yield was higher than those observed by other authors at higher initial stockings (Wise & Pitman, 1981; Sims *et al.*, 1999a; Sims *et al.*, 1999b; Sims *et al.*, 2001). The yield values reported in Chapter IV were determined from commercial-sized plots with conservative assumptions for site quality, and represents the actual potential for biomass production with the current silviculture regimes. Similar average yields are reached in low density plantations, even though there is a delay in obtaining products, but an increase in average tree size (Dickmann, 2006).

7.1.2. Litter

For the aboveground litter, the changes in carbon contained in this compartment with age since afforestation was evaluated (Chapter II), and a model for estimating the C in aboveground biomass and litter for *E. globulus* and *E. nitens* was described (Chapter IV).

The C sequestration rates in the organic layer (0.9-2.9 Mg C ha^{-1} yr^{-1}) were considerably higher that those reported for Central Europe and Scandinavian Countries (Vesterdal *et al.*, 2008; Berg *et al.*, 2009) and lower than in tropical forests (Ostertag *et al.*, 2008). The litter accumulation rate, equilibrium stock and age at which the equilibrium is reached were different in each species. The highest litter accumulation rate was observed in *E. nitens* stands followed by *P. radiata* and finally *E. globulus* stands. The higher litter accumulation in *E. nitens* stands was possibly associated with the particularly high growth rate of this species. However, litter accumulation was lower in the *E. globulus* stands than in *P. radiata* stands, despite the greater increase in biomass, probably due to faster decomposition of eucalypt litter than of *P. radiata* litter, as recorded for the region (Alvarez *et al.*, 2008) and elsewhere (Paul & Polglase, 2004; Lemma *et al.*, 2007b; Huang *et al.*, 2011), and which is attributable to differences in the chemical composition of the litter, as well as to the different climate conditions for plantations of both species.

With the exception of *E. nitens* stands, in which litter accumulation occurred very early on, in *E. globulus* and *P. radiata* stands, development of the litter layer coincided with canopy closure (around 5 yr after establishment). This implied increased litter input, as well as a lower mineralization rate as a consequence of shading. Canopy closure may have resulted in a sharp decrease in root turnover in ground vegetation, which would have been expected to be greater under pine because of the higher degree of shade provided by pines than by eucalypts (González-Hernández *et al.*, 1998; Silva-Pando *et al.*, 2002). On the other hand, the death of ground vegetation as a result of shading in *P. radiata* stands contributes to the aboveground litter. In the steady state, C accumulation in the litter layer was 10, 25 and 51 Mg C ha^{-1} for *E. globulus*, *E. nitens* and *P. radiata* respectively. The accumulation rate reached equilibrium at 10 yr in stands of both *Eucalyptus* species, and C accumulation appeared to still be occurring in the litter in 35-year-old *P. radiata* stands.

The proportion of C accumulation in the litter layer relative to that in the aboveground biomass for ages close to the rotation age was 9.5, 23.4 and 21.4% for *E. globulus*, *E. nitens* and *P. radiata* respectively. At the end of the rotation the litter layer constituted between 3 and 11% of the forest system C pool.

7.1.3. Soil organic carbon

Changes in soil organic carbon (SOC) stock after land use change from pasture to forest plantations was evaluated empirically by direct measurement (Chapter II), and by eco-physiological modelling (Chapter VI). The effect of afforestation on SOC stability was also evaluated (Chapter V).

Mineral soil carbon to a depth of 30 cm accounts for 31, 34 and 22% of the total amount of carbon (aboveground biomass + litter + mineral soil) at the end of the rotation for *E. globulus*, *E. nitens* and *P. radiata* respectively. Nevertheless, the contribution of soil (litter + mineral soil) to the overall C sequestration due to land use change ranged from 8 to 18% (on average, 15%), which is similar to that reported by De Vries *et al.* (2006) and Woodbury *et al.* (2006), in Europe and the United States respectively, and lower than reported by Liski *et al.* (2002). This is because of the relatively small change in soil after land use change, relative to biomass.

The plantations under study were established on pastures growing on soils with rather high SOC contents (74.8 Mg ha^{-1} to a depth of 30 cm, 27.7 Mg ha^{-1} Std. dev.), which were intermediate between the SOC contents of croplands and natural forest soils in the region (Leirós *et al.*, 2000; Merino *et al.*, 2004). The high SOC contents in the pastures are due to long use of low intensive management based on low intensity tillage and addition of slurry, lime and fertilizers, which enhance grass production and therefore root turnover. The experimental design (paired plots and chronosequences) enabled estimation of the changes in SOC following land use change, after correcting for local tendencies.

The general trend observed for the species under study was large losses of SOC in the upper mineral soils during the first years after afforestation, and subsequent gains, which led to recovery of the initial levels after 20 years. This effect was restricted to the upper mineral soil layers (0-15 cm), whereas no significant differences were found in deeper soil layers, possibly because of the heterogeneity of the type of horizon (Table 2.3). The maximum SOC losses in the first 10 years after afforestation in the upper 0-15 cm mineral soil layer depended on the tree species, and constituted 4-38% of the initial C contents. Gains in SOC took place after canopy closure in the stands, and amounted on average up to 3-18% at the end of the rotation, depending on the species. The effects of afforestation on SOC and differences among species are only attributed to tree-induced microclimatic changes, as soil disturbance for plantation establishment was low in all plots.

In comparison with the pine stands, the eucalyptus stands lost less SOM and the period of SOM loss was shorter, which was possibly due to the different dynamics of the litter accumulation

and understory vegetation development in this type of plantations throughout the rotation. Canopy closure occurred within a short period in pine plantations on former pasture land, which greatly reduced the solar radiation from the third year onwards. This reduced the development of understory vegetation and favoured litter accumulation. On the contrary, in plantations of both types of *Eucalyptus*, the higher transfer of crown light promoted the development of understory vegetation, which at the beginning of the rotation consisted of grasses (González-Hernández *et al.*, 1998; Fernández-Núñez *et al.*, 2010), which maintained the period of grass root input longer than in the pine plantations.

Similar patterns to those described above have been described in other studies (summarized in Table 2.6). These studies were selected to represent the same land use change as described in the present study, and to provide information enabling estimates in the short and long-term and at compensation age. In the short term (<20 yr), the average losses were similar to those observed in the present study. The average C compensation ages (age when the original SOC content is reached) recorded in the present study, between 10 and 25 years, were within the most common range reported in the relevant literature, although much longer compensation ages have been simulated in colder climates. Moreover, greater long-term gains (although highly variable) than those observed here have been reported, within a longer time frame than considered in the present study.

With the aim of evaluating the site factors affecting the changes in SOC after afforestation, a specific study was made of the upper and the lower part of the curves representing the average changes. The SOC gains were slightly higher in the stands with higher site indexes, which reflects the influence of higher biomass production on litter production and root turnover. Nevertheless, the effect of site index could not be evaluated accurately, since site index was rather high in most cases. Soil texture is one of the most important factors determining SOC dynamics (Mendham *et al.*, 2003). However, the present study, did not identify any changes in the SOC dynamics attributable to soil texture, probably because the soils were rather homogeneous with regard to this parameter. Moreover, it was also seen that the losses were greater and later recoveries higher at high initial SOC. This effect has also been reported by other authors (Knops & Tilman, 2000), although in the present study, significant differences were only observed for the *Eucalyptus* species. This was probably because of high shading in *P. radiata* plots, in which large losses of SOC occur in all stands, independently of the initial SOC density.

Moreover, the C/N ratios in the mineral soil layer of the mature afforested soils under pines increased throughout the rotation, as previously reported (Smethurst & Sadanandan Nambiar, 1995; Giddens *et al.*, 1997; Jug *et al.*, 1999; Ussiri *et al.*, 2006), probably because of the greater influence of forest litter on SOM quality throughout the rotation. This would reflect a shift from organic input dominated by grass litter, to forest litter containing larger amounts of recalcitrant biopolymers (resins, waxes, suberin and cutin-derived compounds (Chefetz *et al.*, 2002; Otto & Simpson, 2006)). However, no changes with age since afforestation were observed in the C/N ratio in the eucalyptus stands, possibly because of the presence of more grass in the underground

vegetation in these plantations. The subsequent analysis of SOM revealed that the soil under eucalypts contained more carbohydrates. In addition, the time frame must be considered, since in the period studied for eucalypts (0-20 years) there were also no differences in the C/N ratio in the *P. radiata* plantations.

Regarding SOM stability, initial losses did not only affect the more labile organic SOM compounds, but also complex and recalcitrant C compounds, as previously reported (Fierer *et al.*, 2003; Goberna *et al.*, 2006; von Lützow *et al.*, 2006; Dorodnikov *et al.*, 2007). ^{13}C CP-MAS NMR and DSC techniques revealed important losses of easily degradable compounds in *P. radiata* stands, such as carbohydrates, carbonyl/carboxyl groups, and even aliphatic and aromatic components, after afforestation (Chapter V). In the oldest stands of the pine chronosequence, there were important losses of aromatic components, which supposedly more resistant to microbial degradation (Baldock *et al.*, 2004). Consistent with these trends, the changes in Φ/RCO_2 values during the first years after afforestation indicate microbial degradation of substrates that are more reduced than carbohydrates. Depletion of this type of compound at the end of the rotation period explains the low Φ/RCO_2, which indicates that carbohydrates were the main C source for microorganisms.

On the other hand, losses of more labile SOC compounds predominated across the study period in *E. globulus* stands. It is possible that the presence of grass, along with the higher litter decomposition rates resulted in greater C inputs in the eucalypt plantations. The litter derived from grass vegetation is dominated by carbohydrates that decompose relatively easily, which would explain the lower loss of SOM and the larger amounts of carbohydrates in these afforested soils. The results show that at the end of the study period for all species, the only source of C in the soil is the fresh carbohydrates derived from litter decomposition, and that the microbial biomass is adapted at that point to that type of substrate. This promotes the accumulation of new aliphatic/aromatic fractions, as shown by the results of the ^{13}C CP-MAS NMR and DSC analyses of the soils at the end of the rotation. The new source of SOM is probably derived directly from the surface layer and the roots, which are directly associated with the forest species used in the afforestation, and may indicate the proximity of the equilibrium between decomposition and incorporation of organic matter.

7.1.4. Products and bioenergy

Carbon accumulation in wood products was evaluated for all species studied (Chapter VI), by taking assumptions for the harvested wood consumption for each industrial use, transformation efficiency, process loss reallocation and average lifespan of transformed products were considered. The balance between new products being made and old products being decomposed, constitute a pool of products-in-use, in which C is retained (Nabuurs & Schelhaas, 2002). This new pool has two mitigatory effects: carbon sequestering in its own matter, and substitution of other products that involve fossil C release during their fabrication. This implies that use of wood

products generates a carbon sink that otherwise would not exist. Since it is not clear as yet how carbon in wood products will be credited in international agreements, it is important to compare the total outcome of the scenarios with and without wood products (de Jong *et al.*, 2007).

Under the assumptions of lifespan considered here, the total C stock in forest products is greater than the average accumulation in biomass for infinite rotations (Table 6.9), with an average stock after 125 years of between 47 and 252 Mg C ha^{-1}, depending on the species used and the management regime applied. As our assumptions regarding the lifespan of products can be considered as conservative relative to the values considered by other authors (Row & Phelps, 1990; Karjalainen *et al.*, 1994; Karjalainen, 1996; Nabuurs & Sikkema, 2001; Karjalainen *et al.*, 2002), it is possible that the C stock in forest products may be even larger.

There are other forest products that must be considered as creating C fluxes rather than as stock, i.e. those destined for bioenergy production. The mitigation potential of bioenergy use is strictly the only such effect possible in forest systems in the long term (Lindner & Karjalainen, 2007), as the other pools considered reach equilibrium after land use change, and the accumulatory effect then becomes limited. The mitigation potential of bioenergy use was evaluated for each species (Chapter VI), taking into account burning of forest waste burned in biomass factories and reuse of wood products at the end of their useful life, as well as use of harvested slash residues for bioenergetic purposes. The mitigation potential of bioenergy use ranged from 0.5 to 4.4 Mg C ha^{-1} yr for all species and management alternatives.

Regarding avoided emissions obtained by bioenergy use, woody biomass can be converted via combustion, gasification, pyrolysis and fermentation, and the energy recovered depends on the conversion technology (McKendry, 2002). There are several ways of estimating the bioenergy production from forest stands. Several models were developed for bioenergy estimation for low density energetic plantations of *E. globulus* and *E. nitens*, depending on the final transformation process (Chapter IV). Predicted energy production values for low density energetic plantations and transformation via combustion ranged between 3.4-3.5 and 4.0-4.1 TJ ha^{-1} for *E. globulus* and *E. nitens* respectively. These values are higher than those reported for other eucalypts (Moreira, 2006). The annual logging residue energy yield was 33-35 and 53-54 GJ ha^{-1} yr^{-1} for *E. globulus* and *E. nitens* respectively, similar to the 65 GJ ha^{-1} yr^{-1} estimated for both species together, also in northern Spain (Pérez *et al.*, 2008). Usable cellulose at the end of the rotation was 83.2-85.0 and 110.9-108.4 Mg ha^{-1} for *E. globulus* and *E. nitens* respectively. *Eucalyptus nitens* provided better results than *E. globulus* considering all ways of expressing bioenergy production.

7.2. Effect of different C estimation approaches

7.2.1. Plot sampling for soil organic carbon estimation

An intensive sampling intensity approach was carried out to evaluate the changes in soil C stock after afforestation on former pasture land, at landscape level (Chapter II). Although the objective was to evaluate the changes in C stock in several pools after land use change from pasture to forest plantations, this enabled evaluation of the usefulness of this empirical approach in evaluating static land use (pasture), as well as a changing system (plantation). The sampling intensity for all species studied was 58.3 km^2 $plot^{-1}$ for each land use considered, in a rather homogeneous region from the point of view of climate and soil properties.

The carbon density in the pasture sub plot depended on the species, although there were not significant differences between species. Variations of between ±10%-±21% were observed for the 95% confidence interval for the pasture sub plots. This demonstrates the high variability in soil carbon density estimation at the landscape level, even in the case of steady state systems and high sampling intensity. Since pasture management and species composition were similar in all plots, the observed variability can be attributed to local differences in environmental conditions. This results show the convenience of correcting for local tendencies by use of the paired plots and chronosequence technique when the aim is to identify the effect of age since afforestation.

The experimental design, based on well-replicated chronosequences combined with paired plots, enabled most of the variability and local conditions to be controlled, and average regional trends in the C dynamics for the three most common tree species used in afforestations in northern Spain to be determined. Despite the rather homogeneous environmental conditions, the C dynamics after afforestation were highly variable, especially in the *Eucalyptus* chronosequences. Such high variability, already reported in other studies at landscape scale (Johnston *et al.*, 1996; Turner & Lambert, 2000; Conant *et al.*, 2003; Poeplau *et al.*, 2011), emphasizes the risk of reaching wrong conclusions about SOC dynamics when the experimental design does not take into account most of the variability.

7.2.2. Effect of bole sampling intensity on the accuracy of tree-level wood estimation

The effect of bole sampling intensity on the individual tree dry mass estimation through ratio type estimators from complete fresh weight and moisture sample disks (*CW*), or from complete cubication and basic density estimations (*PW*) was considered (Chapter III).

The results showed that there may be a high degree of error and that this depends on the sampling intensity, the method used (*CW* or *PW*), the average tree size and the initial (default) sampling point in the stem. Moreover, estimations with both methodologies are known to be biased

(Cunia, 1979; Valentine *et al.*, 1984; Briggs *et al.*, 1987), but in all cases towards overestimation. The overestimation increases with decreases in both sampling intensity and average tree size.

The main source of this error is wood moisture content and basic density change along the stem, which affect the estimation of dry biomass by the *CW* and *PW* methods respectively. Minimum moisture content and basic density occur in the basal part of the stem, which is obviously where most of the accumulated weight and volume occur (Figs. 3.5 and 3.6). This effect must therefore be taken into account with a sufficient and well distributed number of subsamples along the stem. One way of addressing this problem, when taper functions are available, is the density integral approach (Parresol & Thomas, 1989). The weighed average is an alternative method that attaches more importance to those observations in the lower part of the stem, and therefore more closely related to volume.

Chave *et al.* (2001) reported that the biomass values of the smallest trees greatly affect the values of the model parameters in the allometric relation. This effect even occurs when a weighted adjustment methodology is used, because the smallest trees, which are less variable, are more important than the largest trees, because of heteroscedasticity correction. It is therefore advisable to obtain the complete dry weight of the stem of small trees.

A rather intensive sampling scheme should be implemented when relatively high precision is required (Parresol, 1999). The results reported in Chapter III provide guidelines to evaluating the sampling intensity required for a given assumed error when biomass equations are developed for similar species, although specific study of the variation in wood moisture or basic density along the stem is needed. Of the methods considered here, the *CW* method produced better results for the largest dimensional class than the *PW* method. This must be taken into account because the *PW* method is usually used for large trees in which complete weighing is time-consuming.

The results clearly show the trends in relative errors derived from measurement of the bottom disk or the bottom log, considered by default as the first section that should be measured. If systematic sampling is used, it is advisable to establish the sampling intensity before randomizing the position along the stem of the first disk or log to be measured. In the case of the *PW* method, it is not recommended to take only one sample log per tree, although this approach was used here. The sampling intensity should be split along the stem, and it is advisable to obtain a good representation of the bole area where average basic density is likely to be found (between 30 and 35% of total height for *E. nitens*).

7.2.3. Effect of independent variables on development of tree-level biomass equations

The usefulness of crown variables for predicting crown biomass fractions was evaluated (Chapter III). Some crown variables are known to work well as predictors of crown fractions (Clark, 1982; Satoo & Madgwick , 1982; Carvalho & Parresol, 2003). In the present study, inclusion of crown variables (live crown base height, crown diameter and living crown length) improved the

RMSE by 1.8%, 10.8%, 19.1% and 17.3% for dead branches, thick branches, twigs and leaves respectively, in the individual fit. These improvements are smaller than those obtained by António *et al.* (2007) for *E. globulus* in Portugal, probably because of the lower genetic variability in the plantations considered in the present study. The greatest improvement was for leaves, which implies better estimation of a fraction that is very difficult to predict and is very important in nutritional and ecological studies.

The usefulness of stem variables was also evaluated for both crown and stem fractions. In this study, inclusion of total height (*h*), together with diameter at breast height (*d*) only resulted in improved accuracy for wood fraction, although other authors have reported significant improvement for several fractions (Loomis *et al.*, 1966; Pearson *et al.*, 1984; Bartelink, 1996; Reed & Tomé, 1998; Monserud & Marshall, 1999; António *et al.*, 2007). Although diameter at stump height (d_{st}) worked well as a predictor, it is seldom measured in forest inventories. On the other hand, it is sometimes useful to estimate dry biomass when trees have already been felled and only stump dimensions are available (Diéguez-Aranda *et al.*, 2003).

The ability of the fitted biomass equations to evaluate the proportion of each aboveground biomass component for a range of diameters has seldom been studied. The proportions are often considered as parameters in ecophysiological models (Pérez-Cruzado *et al.*, 2011a), particularly for small diameters (Sands & Landsberg, 2002). The present results show that, if a threshold diameter at the small end is considered for defining a wood component, minimum breast height diameter must be considered to define the range of use of the biomass component equations, if sound estimation of these percentages is sought.

7.2.4. Effect of yield tables on CO_2Fix model estimation

The CO_2Fix model was parameterized and used in Chapter VI to evaluate the effects of different management practices on plantations of fast growing species established on former agricultural land, in relation to climate change mitigation. Previous studies using the CO_2Fix model concluded the strong effect of yield tables on model estimation (Nabuurs *et al.*, 2008), which is why the user should choose yield tables that are representative of the stand under study. The effects of alternative models on representative yield estimation tables were evaluated (Chapter VI), by comparing two local forest specific growth models with a specific model for afforestation with *P. radiata* on agricultural land and validating the results with real field measurements.

Although the profitability of selected forest-specific models for *P. radiata* have been found to be good enough for forest land (Castedo-Dorado *et al.*, 2007; Castedo-Dorado *et al.*, 2009), the results obtained here demonstrated a slight bias when the models were applied to forest plantations established on former agricultural land. This may be because of the higher average site quality observed in these plots relative to those used for model fitting. Although the dynamic model (Castedo-Dorado *et al.*, 2007) worked better than the static model (Castedo-Dorado *et al.*, 2009), the best estimations were observed for the specific static models (Figs. 6.2-6.3). CO_2Fix biomass

module estimations have also been validated for the biomass compartment with previously published data (Masera *et al.*, 2003). The latter author also reported underestimation of biomass, which was attributed to the fact that model inputs were derived from managed forest whereas the previously published data was from undisturbed forests.

7.2.5. Sensitivity analysis for branch, root and leaf turnover in CO_2Fix

The sensitivity of eco-physiological models to variations in different parameters must be known because this has implications for model parameterization. When a model is highly sensitive to variations in a given parameter, the parameter must be estimated as accurately as possible, because the effect of small deviations in its estimation has large effects on the final predictive value of the model. Moreover, some parameters are difficult to obtain, because direct measurement implies destructive methodologies or very time consuming techniques. This is the case with leaves, branches and root turnover (*LT*, *BT* and *RT* respectively, Jourdan *et al.*, (2008)), and estimations for CO_2Fix parameterization are usually based on published data.

Nabuurs *et al.* (2008) carried out a sensitivity analysis of the CO_2Fix model for temperate and tropical forests, and concluded that stem parameters (CAI, density and carbon content) and initial carbon stock in type 2 humus, largely determine the outcome of the model. However, the model sensitivity in relation to other parameters, such as *LT*, *BT*, and *RT*, is still not clear (de Wit *et al.*, 2006). A detailed approach is described in Chapter VI for the determination of branch and root turnover from species-specific allometric relations, whereas leaf turnover was estimated from already published 3-PG parameters for the species under study. A sensitivity analysis was carried out for these three parameters considering the long term effects of variations in the parameters on soil C stock.

Sensitivity analysis for the effects of *LT*, *BT* and *RT* on soil C estimation showed that the Yasso model is very robust in relation to these parameters, all of which showed deviations less than ±0.5% with variations up to ±20% in the turnover parameter. The robustness of the Yasso model relative to other soil C models has been studied for other parameters by Palosuo *et al.*, (2008), who concluded that the Yasso model provides more conservative estimates of the changes in SOC after input of fresh organic matter, because of higher decomposition estimation. Moreover, *RT* was the most sensitive parameter, probably because root turnover is the most important source of organic matter to mineral soil in temperate forests (Rasse *et al.*, 2001)

7.2.6. Validation of the Yasso soil carbon model

Since soil carbon density measurement is time consuming and there is a large degree of uncertainty associated with the estimation, the development of adequate models for its estimation is a key factor. Validation of the Yasso model estimation in the climate and growth conditions of fast growing plantations established on former pasture land in southern Europe was evaluated in Chapter VI. The model was parameterized for specific plots and initialized with real data on

pasture SOC content in 120 paired plots, and the model was run until the specific age of each afforested plot, when the real observed SOC content was compared with that predicted by the model.

Results for soil module validation showed that estimated values were close to the observed values for the lowest carbon densities, whereas the highest carbon densities were greatly underestimated (by 40-52%, Fig. 6.4). An age-related trend was observed (Chapter II), in the same plots, with the lowest carbon densities in youngest plots and highest carbon densities in the oldest plots. Bias in model outputs were therefore due to overestimation of decomposition rates by the model, which led to greater differences with increasing time since land use change. This effect has been reported by other authors who parameterized the Yasso model for *Eucalyptus* spp. (Lemma *et al.*, 2007a) and for other species (de Wit *et al.*, 2006; Palosuo *et al.*, 2008). This was attributed to overestimation of decomposition rates in soil compartments, but also because litter input other than trees was not considered.

7.2.7. Methods for evaluating soil organic carbon stability

The stability of SOC was evaluated (Chapter V) by analysing several soil samples from the *E. globulus* and *P. radiata* stands described in Chapter II (selected as representative of the average trends) by ^{13}C CP-MAS NMR and thermal analysis. The changes in SOC throughout the rotation in the afforested stands resulted in changes in the shapes and sizes of the DSC curve, as well as in the temperature of the combustion peaks. The existence of carbohydrates and aliphatic C in the soil was detected by both ^{13}C CP-MAS NMR and DSC techniques. In the DSC curves, these compounds yielded combustion peaks at 325-339 ºC and 380 ºC, respectively. The ^{13}C CP-MAS NMR spectra yielded peaks in the 55-110 and 0-60 ppm regions respectively.

However, the aromatic fraction was not clearly identified, which is attributed to the high C content. These substances appear in the 110-165 ppm region of the ^{13}C CP-MAS NMR spectrum, whereas in the DSC curve, the presence of these compounds should appear as a combustion peak at temperatures higher than 400 ºC (Plante *et al.*, 2009). The high aliphatic contents of the samples under study may have obscured this peak, as the peak combustion of aliphatic and aromatic compounds occurred at similar temperatures, and generated an Exo 2 peak below 400 ºC. The DSC curve area was broader, with a final combustion temperature of about 530 ºC. The final combustion temperature of the samples without the aliphatic fraction was about 502 ºC. On the other hand, soils with low C content yielded spectra with a very broad peak from the carbohydrate to the aromatic region (100-165 ppm, Fig. 5.3). It is well known that it becomes difficult to acquire a ^{13}C CP-MAS NMR spectrum with an acceptable signal-to-noise ratio for low C contents (5 g kg^{-1},Kögel-Knabner, 1997). In such cases, DSC appears to be more sensitive than ^{13}C CP-MAS for differentiating the carbohydrate from the aromatic C.

Therefore, DSC does not provide an accurate relation between the thermal properties and the nature of the SOM in samples with high percentages of C and a high diversity of SOM, although it

distinguishes the carbohydrate from the aromatic fraction in samples with low C content and poor diversity of SOM. This technique also has the advantage of providing reproducible results through simple and rapid experimental phases, avoiding the use of reagents and handling of the soil. According to these observations, application of both ^{13}C CP-MAS NMR and DSC provides more accurate information about the nature of the SOM, because the techniques complement each other when applied to soil samples with a wide range of C contents.

Calorimetry enables continuous monitoring of the basal metabolism, expressed as both heat rate (Φ) and soil respiration (RCO_2). The ratio of these two parameters, Φ/RCO_2, provides useful information about microbial metabolism, in relation to the type of compounds in the SOM. Φ/RCO_2 can directly detect the degradation of aromatic and aliphatic compounds, which in this case enabled interpretation of the observed changes in the DSC curves. The biochemical reactions involved in the oxidation of those macromolecules cannot be monitored by exclusive measurement of the CO_2 rate, because the biochemical pathways used to degrade aliphatic C chains do not release CO_2 during the first phases of the oxidation process, and involve carboxylation reactions, which in some cases release heat but consume CO_2. This may also explain why the correlation between microbial biomass and RCO_2 (R^2 = 0.68) was weaker than that between microbial biomass and Φ (R^2 = 0.94), and also the low correlation between the heat and CO_2 rate reported by other authors (Critter *et al.*, 2004).

The results of the present study show that Φ/RCO_2 is closely associated with the metabolic efficiency of the soil microbial biomass (measured as qCO_2, amount of CO_2 respired per unit of microbial biomass (Thiet *et al.*, 2006)), and with the availability of SOM to soil microorganisms (expressed as C_{mic}-C). This also enables a better understanding of the changes in qCO_2 and SOM.

In conclusion, the calorespirometric ratio, along with other biological parameters, such as qCO_2 and C_{mic}-C, may be a useful tool for studying the patterns of soil microbial metabolism. This parameter works as a complex and sensitive index that provides information about changes in metabolism in response to the nature and redox state of the substrate and to the availability of the SOM to microbial attack, yielding new insight into the biological mechanisms involved in the process.

7.3. Effect of different management practices on carbon in forest stands

7.3.1. Effect of species selection

The effect of species selection on C dynamics after afforestation of former pasture land was studied for biomass, soil, products and bioenergy, in relation to climate change mitigation. Changes in biomass C differed greatly among species, site index and silvicultural management.

The changes in the average C accumulation rate in tree biomass in the measured plots are described in Chapter II. *Eucalyptus nitens* was the most effective species for sequestering C, whereas the highest stock was reached in *P. radiata* stands at the end of the rotation. These results are consistent with those obtained with empirical growth models developed for *E. globulus* and *E. nitens*, described in Chapter IV, and with those obtained by CO_2Fix simulation analysis, described in Chapter VI.

Changes in C in the mineral soil (to a depth of 30 cm) was evaluated in Chapter II, by direct measurement in paired plots established as chronosequeces, for *E. globulus*, *E. nitens* and *P. radiata*. The results revealed important differences in the SOC dynamics following afforestation, attributable to the tree species and the associated vegetation. Although SOC losses were recorded after afforestation with all species, losses were generally lower and the periods of loss were shorter in the soils under the two eucalyptus species. In the *P. radiata* stands there was a clear net gain of SOC from 25 years onwards, because of the longer rotation. In both eucalyptus stands, the compensation ages were close to the end of the rotation, which implies no net gains prior to clearcutting in most cases. Moreover, the variability in SOC in the earlier period (0-10 yr) was much higher in both of the *Eucalyptus* stands than in the *P. radiata* stand (Fig. 2.2). This was probably due to death of the understorey vegetation in the forest subplot, because of shading, which leads to loss of SOC in all *P. radiata* plots, independently of the initial conditions. Changes in C over time since afforestation was also evaluated in Chapter VI, by use of the Yasso model. The results were similar to those obtained by direct evaluation in Chapter II, since simulations for both *Eucalyptus* species remained close to the initial value, whereas increments were predicted for *P. radiata* stands (Fig. 6.6).

The different patterns in the SOC dynamics may be determined by the different SOM dynamics and litter turnover for the three tree species studied. Thus, it is possible that the rapid litter turnover in both types of eucalyptus stands prevented SOC losses in the first years after afforestation, as also suggested by Vesterdal *et al.* (2008), and Huang *et al.* (2011). However, the differences in SOC may also be due to the different ground vegetation development in eucalyptus and pine stands, which affects the SOC via different mechanisms (Lugo & Brown, 1993; Silver *et al.*, 2004). In the pine plantations, the stronger solar radiation crown interception exerts a negative influence on the ground vegetation (very large decreases in the ground vegetation occur from the 5th year (Omil *et al.*, 2007)) and probably also negatively affects litter decomposition. Thus, the higher losses of SOC observed in the young pine plantations may be due to the lower transfer of organic C to the mineral soil, as a consequence of the lower litter inputs from the ground vegetation and the lower decomposition rate of the litter.

In addition to differences in changes in total C after afforestation with the considered species, some chemical differences were also reported. The C/N ratios in the mineral soil layer of the mature afforested soils under pines increased throughout the rotation, but remained constant in the *Eucalyptus* stands. This was probably due to the greater presence of grass in the underground vegetation in *Eucalyptus* plantations than in the *Pinus* plantations. Moreover, the production of

recalcitrant compounds from crown litter and their release to the mineral soil may explain the higher C/N ratio in the soils under mature pine plantations. The higher C/N ratio in the afforested soils may also be due to a lower presence of legumes in the understory vegetation (Corbeels *et al.*, 2002) and to higher N immobilization in trees.

The stability of SOC was evaluated for *E. globulus* and *P. radiata* stands afforested on former pasture land and established as a chronosequence (Chapter V). Application of ^{13}C CP-MAS NMR and DSC revealed important losses of easily degradable compounds, such as carbohydrates, carbonyl/carboxyl groups, and even aliphatic and aromatic components after afforestation, as well as the degradation of more reduced substrates. Large losses of the aromatic components were observed in the oldest stands of the pine chronosequence, and probably occurred in the years following land use change. The low Φ/RCO_2 in the oldest plots indicates that carbohydrates were the main C source for microorganisms, possibly those produced by plant decomposition.

For the other parameters studied here, *E. nitens* was the most effective species for sequestering carbon in products, and for bioenergy (Chapter VI), as well as for energetic yield and usable cellulose (Chapter IV). For the no slash harvesting scenario, both *Eucalyptus* species showed similar mitigation potential through bioenergy use (Table 6.9).

7.3.2. Effect of rotation age

Evaluation of the effect of lengthening the rotation period was carried out in Chapter II. Rotation age affects the total amount of carbon accumulated at the end of the rotation, as well as the accumulation rate. Prolongation of the rotation age by 10 and 5 years for *Eucalyptus* and *P. radiata* respectively, relative to the reference rotation age for each species (10 years and 30 years for the *Eucalyptus* spp. and *P. radiata* respectively) resulted in a C sequestration rate ranging between 11.7 and 14.6 Mg C ha^{-1} yr^{-1} for all species (average value for the three species, 12.7 Mg C ha^{-1} yr^{-1}, Table 2.5). Considering that the area afforested in northern Spain with these three species is estimated to be 135000 ha for the period 1994-2006 (MAPA, 2006), these accumulation rates imply a sink of 1.6-2.3 Tg C yr^{-1} (average 1.7 Tg C yr^{-1}) at the end of the rotation. Therefore, lengthening of the rotation by 100% and 16.6% for *Eucalyptus* and *P. radiata* respectively, supposes an increase in the average accumulation rate for the first rotation of 38.2, 16.0 and 1.2% relative to the reference rotation age for *E. globulus*, *E. nitens* and *P. radiata* respectively. These and previous results (Balboa-Murias *et al.*, 2006; Diaz-Balteiro *et al.*, 2009) show that in order to maximize the C sink capacity, plantations should be managed according to the optimal harvesting schedules for these species. In the present study, the results show that the C sink capacity of these plantations can be increased greatly by prolonging the rotation period.

It must be considered that the results of Chapter II correspond to an average trend for the observed plots, and it is difficult with this information to predict the changes in a forest stand and the age of mean annual increment. Nevertheless, the results are consistent with those obtained by application of the empirical models previously in use in the region (Fernández López, 1982;

Rodríguez *et al.*, 2002; García & Ruiz, 2003; Diéguez-Aranda *et al.*, 2005; Castedo-Dorado *et al.*, 2007; Castedo-Dorado *et al.*, 2009). Although there is not a more accurate way of estimating the soil changes, more detailed studies through models developed in several parts of this doctoral study can be applied to rotation length optimization considering sequestration estimation in biomass, products and bioenergy substitution. The decision on rotation age should be considered as being directly linked to the silvicultural regime, although financial criteria are generally considered in establishing the optimal rotation for fast growing plantations. The financially optimal rotation produces small dimension wood predominantly for use in the pulp and chipboard industry, which finally leads to a shorter lifespan of harvested wood products.

7.3.3. Effect of silvicultural regime

The effect of different management regimes on climate change mitigation was assessed in Chapters IV and VI. Management regimes aimed at woodchip production (A) and sawn wood production (B) were compared in Chapter VI for *E. globulus*, *E. nitens* and *P. radiata*, and two different initial stocking for *Eucalyptus* short rotation woody crops were evaluated in Chapter IV.

Management alternatives A and B were evaluated by simulation with the CO_2Fix model, considering three site indexes for alternative A and the two highest site indexes for alternative B. Four pools -biomass, soil, products and bioenergy- were considered for comparison of the two alternatives in relation to climate change mitigation. When rotations were simulated for up to 200 years, the average stock in biomass, soil and products reaches an equilibrium between inputs (biomass growth, slash and living biomass turnover and thinning and clearcutting, respectively), and outputs (thinning and clearcutting, soil respiration and wood discarded at the end of its useful life, respectively), as reported by other authors (Apps *et al.*, 2000; Nabuurs & Schelhaas, 2002). For this, the average stock was estimated for a period of 0-200 years for biomass, and after the steady state was reached in the other pools, at 50 yr for soil and 125 years for products. Nevertheless, the mitigation potential of bioenergy use can be considered as an annual rate of avoided emissions, because although the effect is immediate after bioenergy use, the avoided emissions are contained in a compartment in which the C has been sequestered for thousands of years. In fact, it is the only strict mitigatory effect in forest systems in the long term (Lindner & Karjalainen, 2007), since the other pools considered reach equilibrium after land use change, and the accumulation effect then becomes limited.

Comparing both alternatives considered, the largest C stocks in biomass were reached for all species considered in alternative A, because biomass reduction by thinning takes place in alternative B (Fig.6.6; Table 6.9). The average C stock in biomass in alternative A was between 7-44% and 17-70% higher than in alternative B for the intermediate and high site indexes respectively. These results are consistent with those of Mund & Schulze (2006), who reported that the total amount of carbon in the system decreased as management intensity increased for *Fagus sylvatica* stands in Germany.

For the soil C stock in the steady state, there were slight differences for the different site indexes considered and the management alternatives. For all species the soil C stock increased with site index. Comparing the two alternatives, the C stock was higher in the steady state in alternative B, both when harvesting of 50% of slash residues and no harvesting of slash residues were considered. This was because of incorporation of more organic matter into the soil during pruning and thinning operations. Alternative B supposed an increase with respect to alternative A, of between 0.3-3.4% and 0.4-4.3% for the medium and high site indexes when harvesting of 50% of slash residues were considered, and between 0.2-5.6% and 1.1-6.9% for the medium and high site indexes when no harvesting of slash residues was considered.

Under the assumptions made in Chapter VI, alternative A generates more C stock in the products than alternative B. This may be due to the difference in the assumptions in relation to the lifespan, since a larger proportion of long-term products are generated in management alternative B than in alternative A. The average C stock in products in alternative A was between 24-57% and 25-52% higher than in alternative B for the medium and high site index respectively. Moreover, other effects may have been derived from the silviculture regime, with regard to product lifespan, as a result of the average tree size harvested (Profft *et al.*, 2009), which was taken into account in the present study in terms of the different proportions destined to different industries depending on the average tree size harvested.

The mitigation potential of bioenergy use was higher in alternative A than in alternative B, although more frequent energy harvesting was simulated in alternative B. This follows the same trend as product stock, because part of the energy use is related to transformation of wastes and recycling processes in the log-wood and pulp-wood industry, and as well as slash harvesting. Alternative A supposed an increase with respect to alternative B of 14-77% for the two highest site indexes when harvesting of 50% of slash residues was considered, and of 25-100% and 21-89% for the medium and high site indexes when no harvesting of slash residues was considered. The values obtained for the bioenergy substitution are higher than those reported by de Jong *et al.* (2007) for the bioenergy scenario, which resulted in continuous stream of about 1.36 Mg C ha^{-1} yr^{-1}, even in the case of no slash harvest.

As previously reported, bioenergy management alternatives were only evaluated for the two *Eucalyptus* species (Chapter IV), and two initial stand densities were simulated, 1600 and 2400 stems ha^{-1}. The simulated rotation ages were longer than the average lengths considered for short rotation crops. This practice has some positive effects, such as lower wood:bark ratios and larger average tree sizes. Longer rotations are also a good option if the goal is product flexibility (Dickmann, 2006). The results described in Chapter IV for the single stem rotation showed that 50% increments in initial density resulted in only marginal increases in yield, as well as decreases in rotation, as previously observed (Whitesell *et al.*, 1992). Together with the observations made in Chapter II as regards the compensation age for the soil compartment, short rotation ages and high initial stocking are not recommended when the objective is either bioenergy production or carbon sequestration in *Eucalyptus* plantations.

7.3.4. Effect of slash harvesting

The effect of harvesting slash residues left in the field was assessed in Chapter VI. This management practice affects the soil C stock equilibrium in the steady state, as well as the mitigation potential of bioenergy substitution.

The intensity of the effect of slash harvesting on the mitigation potential of bioenergy substitution is shown in Table 6.9; increments of between 17.8% and 40.3% occurred in response to harvesting 50% of slash relative to no slash harvesting for all species considered. On the other hand, harvesting 50% of slash resulted in decreases in soil C equilibrium stock of between 4.4% and 8.0%. Other authors have also reported no significant C losses after slash harvesting (Johnson & Curtis, 2001). Studies of the global carbon cycle in bioenergy systems conclude that although there may be some decrease in soil carbon associated with biomass production, this is negligible in comparison with the contribution of bioenergy systems towards greenhouse gas mitigation through avoided fossil fuel emissions (Cowie *et al.*, 2006). Regarding the effect of site index, the mitigation potential increased with site index.

Palosuo *et al.* (2008) compared several biomass extraction scenarios with two alternative modelling approaches, and concluded that the intensified biomass extraction slightly decreased the simulated growth of forests and thus the biomass carbon stock and litter input to the soil. This was because yield estimation by the model used (the EFIMOD eco-physiological model) was affected by the intensity of residue extraction. The use of models such as those developed in this study assumes that forest growth is not affected by extraction of residues, although this has been reported to occur (Palosuo *et al.*, 2008). The same effect was observed by (Helmisaari *et al.*, 2011), and could be avoided by returning nutrients to the forest through biomass ash fertilization (Solla-Gullón *et al.*, 2008; Pérez-Cruzado *et al.*, 2011b), which also increases the carbon content because of the charcoal contained in the ash (Santalla *et al.*, 2011).

Logging residues left in the field after first thinning are strongly affected by the top diameter threshold considered (Räisänen & Nurmi, in press), with slash production almost doubled with increments of 2 cm in the top diameter threshold. Therefore there was probably a high degree of uncertainty associated with estimation of slash residues. However, with the current slash harvesting machinery it is not possible to harvest more than 50% of harvest residues (Tolosana, per. com.). A combination of bioenergy and carbon sequestration will be the best option for mitigating CO_2 emissions in the long term, as already reported for plantation management in other countries (de Jong *et al.*, 2007; Kaul *et al.*, 2010).

7.4. References

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7.5. General conclusions

The main conclusions reached in this thesis are as follows:

Chapter II

- ✓ An intensive sampling scheme was used to assess the C sink capacity of stands of the three tree species most commonly used in afforestation programmes in northern Spain. The high spatial variability in the different compartments illustrates the risk of reaching wrong conclusions about SOC dynamics when the experimental design does not cover most of the variability.
- ✓ Afforestation of former pasture land in temperate climate regions resulted in C accumulation rates as high as 11-15 Mg C ha^{-1} in the total systems in the first 20 yr, depending on the species and the rotation length. The results of the study show how selection of the tree species is a major factor influencing the post afforestation C sink capacity, affecting the amounts of C accumulated in both biomass and soil.
- ✓ The role of the tree species is particularly important during the first years after afforestation when the litter input from herbaceous vegetation may compensate for SOC losses. The patterns of SOC dynamics differed greatly in relation to the different tree species used in the afforestation, and were determined by transfer of C to the soil via the ground vegetation roots and by the rate of litter turnover. Both of these sources were lower in the pine plantations than in the eucalyptus plantations, which may explain the higher SOC during the first years after afforestation.
- ✓ The humid temperate climate, along with the lack of physically protected SOM (sandy loam texture of the soils) favoured important losses of SOC in the uppermost mineral soils during the first years.
- ✓ The study provides accurate information on the success of these afforestation programmes as regards CO_2 mitigation. To enhance the C sink capacity, plantations should be managed according to optimal harvesting schedules for the species. Elongation of the rotation length led to larger C sink capacities in all three species. This is especially important in such intensively managed plantations, in which harvesting in short rotations may lead to continuous loss of SOC.

Chapter III

- ✓ Stem sampling intensity has a major effect on dry mass estimation when ratio type estimators and systematic sampling methods are used. This effect depended on the tree

size, and errors increased with decreasing tree size, for a given sampling intensity. This implies that the total dry weight of small trees must be obtained for reasonable estimates.

- ✓ Variation in moisture content and basic density along the stem account for the higher variations observed. Since both accumulated dry mass and volume are accumulated in the lower part of the stems, this part must be properly represented.
- ✓ When the dry mass of an individual tree was estimated from complete bole fresh weight and moisture content of sample disks taken along stem (*CW*), the sampling intensity that guaranteed ±5% of relative difference ranged between 0.75-0.95 disks m^{-1}, depending on the average tree size.
- ✓ When dry mass of individual tree was estimated form bole cubication and basic density samples taken along the stem (*PW*), an unacceptably high sampling intensity was required for ±5% of relative difference estimation.
- ✓ The average basic density usually occurred at a relative height of 30-35% along the stem.
- ✓ Overestimations occurred when systematic sampling is applied, independently of the approach (*CW* or *PW*), although the overestimation decreased as sampling intensity increased.
- ✓ Default consideration of the stump height section resulted in overestimation in the *CW* methodology, whereas underestimations were observed for *PW* methodology.
- ✓ Inclusion of crown variables as predictors in biomass equations models did not improve the estimation for total biomass, wood and thin branches, but did improve the accuracy of estimation for twigs, leaves, thick branches and dead branches.

Chapter IV

- ✓ An accurate tool for crop yield estimation in terms of oven dry mass, total energy, biomass and litter carbon and usable cellulose was provided for the single stem rotation of *E. globulus* and *E. nitens* plantations.
- ✓ Some constraints regarding tree size limitation can be adequately assessed by use of the proposed models, such as threshold diameter at stump height for a given harvesting machinery or self thinning mortality.
- ✓ A 50% increase in the density relative to an initial stocking rate of 1600 stems ha^{-1} resulted in only marginal increases in yield, and decreases in rotation age.
- ✓ The observed yields for *E. globulus* and *E. nitens* planted at densities of 1600-2400 stems ha^{-1} in single stem rotation were similar to those observed at the higher densities usually used in SRF.
- ✓ *E. nitens* displayed faster diametric growth than *E. globulus* for the same initial stocking.
- ✓ Potential annual energy production ranged from 233-245 and 345-364 GJ ha^{-1} yr^{-1} for *E. globulus* and *E. nitens* respectively.
- ✓ Although specific consumption of *E. nitens* is higher than for *E. globulus*, usable cellulose is 37-42% higher in *E. nitens* than in *E. globulus* plantations.

- ✓ Wood proportion in the stem reached an asymptotic value of 90% when the quadratic mean diameter reached a value of 15 and 20 cm for *E. globulus* and *E.nitnens* respectively.

Chapter V

- ✓ Thermal and calorimetry techniques proved to be valuable tools for determining the main changes brought by afforestation, in relation to the nature of the SOM and microbial metabolism.
- ✓ ^{13}C CP-MAS NMR and DSC provide useful information about the composition of the SOM because they complement each other when applied to soil samples with wide ranges of C contents.
- ✓ The relationship between SOM composition and microbial metabolism was successfully determined by isothermal calorimetry, by means of the calorespirometric ratio, along with other biological parameters obtained by this technique, such as qCO_2 and C_{mic}/SOC.
- ✓ The calorespirometric ratio was found to be a sensitive index that provided information about changes in metabolism in response to the nature and redox state of the substrate and to the availability of the SOM to microbial attack.
- ✓ The proposed methodology distinguished differences in the SOM dynamics and microbial metabolism attributable to the tree species, through different understorey vegetation and litter accumulation composition.
- ✓ The humid temperate climate, and perhaps the lack of stabilization in mineral-associated forms and in fine pores, favoured large losses of SOM after afforestation, which even affected highly recalcitrant aromatic compounds.
- ✓ The changes in SOM composition led to alterations in the pattern of microbial metabolism, the efficiency of which was particularly affected by the redox state and energy of the substrates.

Chapter VI

- ✓ The yield tables used have a strong effect on CO_2Fix estimation. The most accurate models, which are as locally relevant as possible models must therefore be considered.
- ✓ When afforestation on former agricultural land is simulated, specific models for these conditions are required.
- ✓ The Yasso model produced increasingly large underestimates of litter and SOC as time since land use change increased. This was due to overestimation of decomposition rates.
- ✓ The Yasso model showed low sensitivity to changes in leaf, branch and root turnover in soil organic matter accumulation estimation.
- ✓ C stock in the products in the steady state supposes a higher pool than for average stock through rotation in living biomass, considering conservative assumptions of product lifespan.

- ✓ Site index is an important factor in changes in system carbon, with variations between ±12% to ±64% for biomass, ±9% to ±13% for soil, ±44% to ±62% for products, and ±45% to ±150% for bioenergy substitution.
- ✓ The pulp-wood management alternative produces higher C equilibrium stocks in biomass and products than sawn-wood alternative, and the C mitigation potential associated with bioenergy use is also higher in the pulp-wood alternative.
- ✓ Harvesting of 50% of slash residues supposes an increase of 17.8-27.9% in the C mitigation rate produced by bioenergy use, but results in a decrease in the soil C stock in the steady state of between 4.4-8.0%.